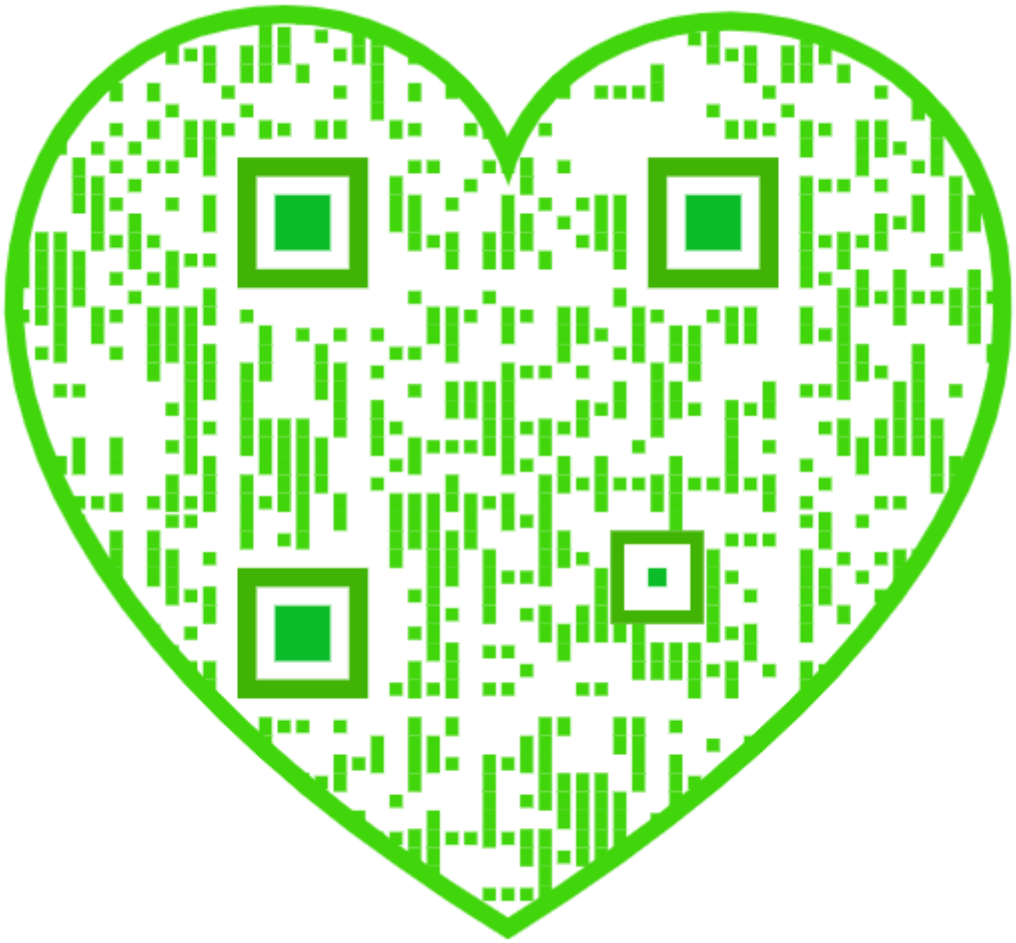

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CLINICAL DIAGNOSIS.

CLINICAL DIAGNOSIS:

THE

BACTERIOLOGICAL, CHEMICAL, AND MICROSCOPICAL EVIDENCE OF DISEASE.

BY

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Vorrede zur englischen Ausgabe.

Indem nun dieses Buch den englischen Collegen in Ihrer Muttersprache zugänglich wird, kann ich nur den Wunsch ihm auf seinen Weg mitgeben, dass ihm auch dort jene günstige Beurtheilung zu Theil wird, der es sich in seinem deutschen Gewande erfreut hat und erfreut.

Möge es ihm gelingen, sich auch in England einen zalreichen Freundeskreis zu erobern!

Herrn Dr. James Cagney, welcher nach der 2ten deutschen Auflage mit grosser Ansicht und grossen Fleiss die Uebersetzung besorgt hat, fühle ich mich verpflichtet für seine Mühewaltung meinen besten Dank zu sagen.

PROF. R. v. JAKSCH.

PRAG, *im Februar*, 1890.

Author's Preface to the English Version.

Now that this book is given to my English colleagues in their own tongue, I can but send it on its way with a wish that it may meet with the same favour that it has had, and still enjoys, in its German dress.

May it be so fortunate as to secure a large circle of friends in England also!

To Dr. James Cagney, who has translated the Second German Edition with great care and assiduity, I feel that my best thanks are due.

PROF. R. v. JAKSCH.

PRAGUE, *February*, 1890.

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TRANSLATOR'S PREFACE.

THE production in another language of a book which has earned a great reputation in its own is a task of some responsibility. My main object throughout has been to follow the author's method, and to convey his meaning as correctly as I could; but I have allowed myself so much freedom as seemed to be consistent with this, and, in one way or another, desirable.

For the new matter I am alone responsible. It is in every instance enclosed within square brackets [ ]. I have collected the references and placed them in order at the end; they thus form a very full Bibliography. The Appendix by Professor Stirling will be of great advantage to the English reader.

I have to acknowledge the courtesy of Professor v. Jaksch, who sent me the advanced sheets of the Second German Edition as they left the press, and his kindness in answering many inquiries. I am indebted to Dr. Robert Maguire for much assistance, and also to Mr. Edgar Crookshank and Dr. Luff. My thanks are especially due to Professor Stirling for the trouble which he has taken in revising the proofs.

Finally, I would testify my gratitude to the publishers, Messrs. Charles Griffin & Company, for the care with which they have prepared the volume for the press, and for the substantial aid which they have given me.

JAMES CAGNEY.

WELBECK STREET, W.,
March, 1890.

TABLE OF CONTENTS.

CHAPTER I.

THE BLOOD.

PAGE

I. Colour of the Blood 1

II. Reaction 2

III. Specific Gravity 4

IV. Changes in the Formed Elements of the Blood . . . 5

1. Oligocythæmia 6

(*a.*) Thoma-Zeiss apparatus for counting blood-corpuscles . 8

(*b.*) Gowers' hæmacytometer 11

(*c.*) Bizzozero's chromocytometer 12

(*d.*) V. Fleischl's hæmometer 13

(*e.*) Hénocque's hæmatoscope 15

2. Leucocytosis 19

3. Leukæmia 20

4. Melanæmia 23

5. Microcythæmia 23

6. Poikilocytosis 24

7. Changes in the formed elements of the blood in chlorosis . 25

8. Changes in the formed elements of the blood in pernicious anæmia 25

V. The Parasites of the Blood 26

A. Vegetable Parasites 26

Method of examining the blood for micro-organisms . . 27

1. Bacillus of anthrax 29

2. Spirillum of relapsing fever 30

3. Bacillus of tubercle 32

4. Bacillus of glanders 32

5. Bacillus of typhoid fever 33

6. Streptococci 33

7. Micro-organisms of the blood in hydrophobia . . 34

8. Bacillus of tetanus 34

9. Bacillus and plasmodium malariæ 34

B. Animal Parasites (*Hæmatozoa*) 35

1. Distoma hæmatobium 35

2. Filaria sanguinis hominis 36

VI. The Chemical Changes in the Blood 37

1. Colouring-matter of the blood 37

1. Blood changes in dyspnœa 41

2. Blood changes in carbonic oxide poisoning . . . 41

PAGE

3. Blood changes in poisoning with sulphuretted hydrogen 42
4. Blood changes in prussic acid poisoning . . . 42
5. Blood changes in poisoning with chlorate of potash . 42
6. Blood changes in poisoning with nitrobenzol . . 43
7. Hæmoglobinæmia 43
8. Recognition of changes in the colouring-matter of the blood 43

2. Proteids 45
3. Urea 46
4. Uric acid 47
5. Sugar (*melithæmia*) 47
6. Organic acids (*lipacidæmia*) 49
7. Lipæmia 49
8. Cholæmia 49
9. Uræmia 51
10. Ammoniæmia 51
11. Acetonæmia 51
12. Changes in the salts of the blood 51

CHAPTER II.

THE BUCCAL SECRETION.

I. Naked-Eye Appearances of the Saliva 53
II. Microscopical Appearances 53
1. Salivary corpuscles 53
2. Red blood-corpuscles 53
3. Epithelium 53
4. Fungi 53
III. Chemical Constitution of the Buccal Secretion . . 55
IV. Constitution of Morbid Saliva in General . . . 56
V. The Sulphocyanide of the Saliva 57
VI. The Saliva in Special Diseases 58
1. Catarrhal stomatitis 58
2. Other forms of stomatitis (mercurial, scorbutic, &c.) . 58
3. Thrush 58
VII. Deposit on Teeth 59
VIII. Coating of the Tongue 60
IX. Coating of the Tonsils 61
1. Croupous and diphtheritic tonsillitis 61
2. Pharyngomycosis leptothricia 61

CHAPTER III.

THE NASAL SECRETION.

I. Naked-Eye and Microscopical Characters—Chemical Constitution 63
II. The Secretion in Affections of the Nasal Cavities . 63

CHAPTER IV.

THE SPUTUM.

PAGE

I. Naked-Eye Characters of the Sputum 65

II. Microscopical Examination of the Sputum 66

1. White blood-corpuscles 66
2. Red blood-corpuscles 66
3. Epithelium 67
4. Elastic fibres 68
5. Spirals 69
6. Fibrinous coagula 71
7. Connective tissue 72
8. Corpora amylacea 72
9. Parasites 73
   1. Fungi 73
      (*a.*) Non-pathogenic 73
         1. Moulds 73
         2. Yeasts 74
         3. Fission-fungi 74
            1. Sarcina pulmonis 74
            2. Leptothrix 74
            3. Bacilli and Micrococci 74
      (*b.*) Pathogenic 75
         1. Tubercle bacillus 75
            Detection of tubercle bacillus . . . 75
         2. Pneumonia microbes 78
         3. Actinomyces 79
   2. Infusoria 80
   3. Vermes 80
10. Crystals 81
   1. Charcot-Leyden crystals 81
   2. Hæmatoidin crystals 81
   3. Cholesterin crystals 82
   4. Fat-crystals (margarine needles) 82
   5. Tyrosin crystals 83
   6. Oxalate of lime 83
   7. Triple phosphate 83

III. Chemical Examination 84

1. Proteids and allied substances 84
2. Volatile fatty acids 84
3. Glycogen 84
4. Ferment 84
5. Inorganic constituents 84

IV. The Character and Constitution of the Sputum in the Principal Diseases of the Lungs and Bronchi . 85

1. Diseases of the bronchi 85
   1. Acute bronchitis 85
   2. Chronic bronchitis and bronchiectasis . . . 85
   3. Putrid bronchitis 85
   4. Plastic bronchitis 86
2. Diseases of the lung tissue 86
   1. Tuberculosis 86
      (*a.*) Miliary tuberculosis 86
      (*b.*) Acute tubercular infiltration 86
      (*c.*) Chronic pulmonary tuberculosis 86

PAGE

2. Chronic (non-tubercular) inflammation of the lung . 87
3. Croupous pneumonia 88
4. Pulmonary abscess 90
5. Gangrene of lung 91
6. Pulmonary œdema 91
7. Hæmoptysis 91
8. Hæmorrhagic infarction 92
9. Pneumoconiosis 92
(*a.*) Anthracosis of the lung 92
(*b.*) Siderosis pulmonum 92
(*c.*) Mason's lung 92

CHAPTER V.

THE GASTRIC JUICE AND VOMIT.

I. Examination of the Gastric Juice 93
1. Naked-eye characters 93
2. Formed elements 93
3. To obtain the gastric juice 93
4. Chemical constituents of the gastric juice 94
1. Pepsin 94
(*a.*) Detection 94
(*b.*) Estimation 95
2. Milk-curdling ferment 95
3. Acids 95
(*a.*) Acidity 96
(*b.*) Hydrochloric acid 96
A. Detection of Free Hydrochloric Acid . . 96
1. *Mohr's* tests 97
2. Aniline-dye tests 97
(*a.*) Methyl-aniline violet reaction . 97
(*b.*) Tropæolin 97
(*c.*) Fuchsin 98
(*d.*) Emerald-green 98
(*e.*) Congo-red 98
(*f.*) Phloro-glucin and vanillin . . 98
(*g.*) Benzo-purpurin 99
3. *Uffelmann's* tests 100
4. Ultramarine and zinc sulphide . . 100
B. Quantitative Estimation of Free Hydrochloric Acid 101
C. Detection of the Organic Acids of the Gastric Juice 103
4. Proteids 103
5. Urea 105
6. Ammonia 105
7. Carbohydrates 105
5. Estimation of the rate of absorption of the gastric contents 106
6. A summary of the chemical examination of the gastric contents 106

II. Examination of the Vomit 106
Naked-eye appearances 107
Microscopical appearances 107
1. Acute gastric catarrh 108

PAGE

2. Chronic gastritis and dilatation of the stomach . . . 108
3. Chronic ulcer of the stomach 109
4. Carcinoma of the stomach 110
5. Parasitic affections of the stomach 111
6. Croup and diphtheria 111
7. Fæcal substances in the vomit 111
8. Pus 111
9. Animal parasites 111
10. Constitution of the vomit in poisoning 111
    1. Poisoning with acids 111
        (*a.*) Detection of sulphuric acid 111
        (*b.*) Detection of nitric acid 112
        (*c.*) Detection of oxalic acid 112
    2. Poisoning with alkalies 112
    3. Poisoning with metals and metalloids 113
        (*a.*) Poisoning with salts of lead 113
        (*b*) Poisoning with salts of mercury 114
        (*c.*) Poisoning with salts of copper 115
        (*d.*) Arsenic poisoning 115
        (*e.*) Phosphorus poisoning 116
    4. Poisoning with alkaloids 117
        (*a.*) Morphia poisoning 117
        (*b.*) Poisoning with nicotin 118
        (*c.*) Poisoning with atropin 118
        (*d.*) Poisoning with ptomaines 118
    5. Poisoning with ethylic alcohol 121
    6. Poisoning with chloroform 121
    7. Poisoning with carbolic acid 122
    8. Poisoning with nitrobenzol and aniline . . . 122
    9. Poisoning with prussic acid 123

CHAPTER VI.

THE FÆCES.

I. Naked-Eye Characters of the Fæces 125
II. Microscopical Characters of the Fæces 127
    1. Constituents derived from the food 127
        (*a.*) Vegetable cells 127
        (*b.*) Muscle fibres 127
        (*c.*) Elastic fibres 127
        (*d.*) Areolar tissue 127
        (*e.*) Fat 127
        (*f.*) Starch granules 128
        (*g.*) Coagulated proteids 128
    2. Formed elements derived from the intestinal tract . 129
        1. Red blood-corpuscles 129
        2. Leucocytes 129
        3. Epithelium 129
        4. Detritus 130
    3. Parasites 130
        A. Vegetable Parasites. 130
            1. Non-pathogenic fungi 130
                1. Moulds 130
                2. Yeasts 130
                3. Fission-fungi 131

PAGE

2. Pathogenic fungi 133
1. Cholera bacillus 133
2. Bacillus of cholera nostras 137
Spirillum of cheese 138
3. Bacillus of typhoid fever 138
4. Tubercle bacillus 139
B. Animal Parasites 140
1. Protozoa 140
1. Rhizopoda 140
(*a.*) Monadines 140
(*b.*) Amœba coli 140
2. Sporozoa 141
3. Infusoria 141
1. Cercomonas intestinalis 141
2. Trichomonas intestinalis 142
3. Paramæcium coli 142
2. Vermes 142
Class I. Platodes 142
(*a.*) Cestodes 142
1. Tænia saginata 142
2. Tænia solium 143
3. Tænia nana 144
4. Tænia flavopunctata 145
5. Tænia cucumerina 145
6. Bothriocephalus latus 145
(*b.*) Trematodes 147
1. Distoma hepaticum 147
2. Distoma lanceolatum 147
3. Distoma Rathonisi 148
Class II. Annelides 148
1. Order Nematodes (round worms) . . . 148
(*a.*) Family Ascarides 148
1. Ascaris lumbricoides . . . 148
2. Ascaris mystax 149
3. Oxyuris vermicularis . . . 150
(*β.*) Family Strongylides . . . 150
Anchylostoma duodenale . . 150
(*γ.*) Family Trichotrachelides . . 152
1. Trichocephalus dispar . . 152
2. Trichina spiralis . . . 153
Rhabdonema strongyloides . 154
3. Insects 155
4. Crystals 155
1. Charcot-Leyden crystals 155
2. Hæmatoidin crystals 155
3. Cholesterin 156
4. Fat crystals 156
5. Oxalate of lime 157
6. Carbonate of lime 157
7. Sulphate of calcium 157
8. Phosphate of lime 157
9. Triple phosphate 157
10. Sulphide of bismuth crystals 157

III. Chemical Examination of the Fæces 158
A. Organic Substances 158
1. Mucin 158

PAGE

2. Albumin 158
3. Peptone 159
4. Urea 159
5. Carbohydrates 159
6. Acids 160
(*a.*) Bile acids 160
(*b.*) Volatile fatty acids 160
7. Phenol 162
8. Indol and skatol 162
9. Cholesterin, fats, and non-volatile organic acids . . 162
10. Colouring matters 163
1. Urobilin 163
2. Blood pigment 164
3. Bile pigment 164
11. Intestinal gases 164
12. Ptomaines 164
13. Ferments 164

B. Inorganic Substances 164

IV. Examination of the Meconium 165

V. Character of the Fæces in certain Intestinal Affections . 165

1. Acute intestinal catarrh 165
2. Chronic intestinal catarrh 166
3. Ulcerative enteritis 166
4. Typhoid fever 167
5. Dysentery 167
6. Cholera 168
7. Hæmorrhagic stools 168
8. Acholic stools 169

CHAPTER VII.

EXAMINATION OF THE URINE.

I. Naked-Eye Inspection of the Urine 170

1. Quantity 170
2. Specific gravity 171
3. Colour 172
4. Reaction 174

II. Microscopical Examination of the Urine 174

1. Cellular constituents (organised sediments) of the urine . 175
1. Red blood-corpuscles 175
2. Leucocytes 176
3. Epithelium 177
4. Casts 180
5. Spermatozoa 188
6. Fragments of tumours 188
7. Parasites 189
1. Fungi 189
(*a.*) Non-pathogenic 189
(*b.* Pathogenic 190
2. Infusoria 192
3. Vermes 193
1. Distoma hæmatobium 193
2. Filaria sanguinis hominis 193

PAGE

3. Echinococci 193
4. Eustrongylus gigas 194
5. Ascarides 194
2. Crystalline and amorphous deposits (unorganised sediments) 194
A. Sediments from Acid Urine 195
(*a.*) Crystalline deposits 195
1. Uric acid 195
2. Oxalate of lime 195
3. Bilirubin and hæmatoidin 196
4. Triple phosphate 197
5. Basic phosphate of magnesia 197
6. Neutral phosphate of lime 197
7. Calcium sulphate 198
8. Hippuric acid 198
9. Cystin 198
10. Xanthin 199
11. Tyrosin and leucin 199
12. Soaps of lime and magnesia 201
(*b.*) Amorphous deposits 201
1. Urates 201
2. Oxalate of lime 202
3. Calcium sulphate 202
4. Brown and yellow concretions . . . 202
5. Fat 202
B. Sediments from Alkaline Urine 202
(*a.*) Crystalline deposits 202
1. Triple phosphate 202
2. Indigo 203
3. Urate of ammonia 203
4. Phosphate of magnesia 204
5. Cholesterin 204
(*b.*) Amorphous deposits 204
1. Urate of ammonia 204
2. Basic phosphatic earths 204
3. Carbonates of the alkaline earths . . 204
4. Carbonate of lime 204
5. Indigo 204
3. Urinary concretions 205
4. Foreign bodies in the urine 205
III. Chemical Examination of the Urine 205
A. Organic Substances 205
1. Proteids 205
1. Albuminuria 207
(*a.*) Renal albuminuria 207
(*b.*) Accidental albuminuria 209
Determination of albumin (serum-albumin) . 209
(*α*) Detection 209
(*β*) Estimation 213
2. Peptonuria 217
3. Albumosuria 220
4. Globulinuria 221
5. Fibrinuria 222
6. Hæmaturia 222
7. Hæmoglobinuria 223
8. Mucinuria 223
2. Carbohydrates 224

PAGE

1. Glycosuria 224
(*a.*) Physiological glycosuria 224
(*b.*) Pathological glycosuria 224
(*a*) Transitory glycosuria 224
(*β*) Persistent glycosuria 224
Determination of grape-sugar . . . 225
(*a*) Detection 225
(*β*) Estimation 230
2. Lævulosuria 236
3. Lactosuria 237
4. Inosituria 237
5. Dextrin 237
6. Animal gum 237
3. Choluria 237
4. Urobilinuria 240
5. Æther-sulphuric acids and their derivatives (indigo-blue skatol, phenol, parakresol, pyrocatechin, hydrochinon) and the aromatic oxyacids 241
(*a.*) Indicanuria 241
Detection of indican 242
Estimation 243
(*b.*) Skatoxyl-sulphuric acid 244
(*c.*) Parakresol-, phenol-æther sulphuric acid, paroxyphenyl-acetic acid, and paroxyphenyl-propionic acid 244
Detection of æther-sulphuric acids . . 244
Detection of aromatic oxyacids . . . 244
Estimation of æther-sulphuric acids . . 245
Estimation of phenols 246
(*d.*) Pyrocatechin 247
(*e.*) Hydrochinon 248
6. Melanuria 249
7. Acetonuria 249
8. Diaceturia 250
9. Lipaciduria 251
10. Lipuria 251
11. Chyluria 252
12. Oxaluria 252
13. Cystinuria 253
14. Uric acid diathesis 253
15. Urea 256
16. Kreatinin 260
17. Ptomaines (putrefaction bases) . . . 262
18. Ferments of the urine 264
B. Inorganic Substances 265
1. Chlorides 265
2. Sulphates 267
3. Phosphates 268
4. Carbonates 271
5. Nitrates and nitrites 271
6. Sulphuretted hydrogen (hydrothionuria) . . 271
7. Peroxide of hydrogen 272
8. Gases of the urine 272

IV. Characters of the Urine in Disease 272
1. The urine in febrile states 272
2. The urine in disorders of the circulation (congestion) . 273
3. The urine in diseases of the urinary organs 274

PAGE

1. Renal affections 274
   (*a.*) Acute nephritis 274
   (*b.*) Chronic nephritis 274
   (*c.*) Contracted kidney 275
   (*d.*) Amyloid kidney 275
   (*e.*) Uræmia 275
2. Pyelitis calculosa 276
3. Cystitis 276
4. Tuberculosis of urinary organs 277
5. Calculus and tumours of the bladder 277
6. Catarrhal urethritis 277
7. Gonorrhœal urethritis 277

4. The urine in diseases of the alimentary canal . . . 278
5. The urine in hepatic affections 278
6. The urine in diabetes mellitus 279
7. The urine in diabetes insipidus 280
8. The urine in anæmia 280
9. The urine of toxic states 281
   1. Poisoning with acids 281
   2. Poisoning with alkalies 281
   3. Poisoning with metals and metalloids 281
      (*a.*) Lead salts 281
      (*b.*) Salts of mercury 281
      (*c.*) Salts of copper 282
      (*d.*) Arsenic 282
      (*e.*) Phosphorus 283
   4. Poisoning with alkaloids 283
   5. Poisoning with ethylic alcohol 283
   6. Poisoning with chloroform 284
   7. Poisoning with carbolic acid 284
   8. Poisoning with nitro-benzol and aniline . . . 285
   9. Poisoning with carbonic oxide gas 285

V. The Detection of Certain Drugs in the Urine . . . 285
   1. Iodoform and salts of iodine and bromine 285
   2. Salicylates 286
   3. Quinine, kairin, antipyrin, thallin, antifebrin, and phenacetin 286
   4. Chrysophanic acid 288
   5. Santonin 288
   6. Tannin 288
   7. Naphthalin 288
   8. Copaiba balsam 289

CHAPTER VIII.

INVESTIGATION OF EXUDATIONS, TRANSUDATIONS, AND CYSTIC FLUIDS.

A. Exudations 290
1. Purulent exudations 290

I. Naked-Eye Appearances 290

II. Microscopical Characters 291
   1. White and red blood-corpuscles and epithelium . 291
   2. Fungi 291

PAGE

1. Micrococci 291
2. Tubercle bacillus 292
3. Bacillus of syphilis 292
4. Actinomyces 293
5. Bacillus of glanders 296
6. Bacillus of anthrax 296
7. Bacillus of leprosy 297

3. Infusoria 298
4. Vermes 298
5. Crystals 298
1. Cholesterin crystals 298
2. Hæmatoidin 298
3. Fat needles 298
4. Triple phosphate crystals 298

III. Chemical Examination of Pus 298

2. Sero-purulent exudations 299
3. Putrid exudations 299
4. Hæmorrhagic exudations 300
5. Serous exudations 300
6. Chylous exudations 301

B. Transudations 301

C. Contents of Cysts 302
1. Hydatid cysts 302
2. Ovarian cysts 302
3. Cystic kidney 304

D. Secretions from Fistulæ 304

CHAPTER IX.

THE SECRETIONS OF THE GENITAL ORGANS.

I. The Seminal Fluid 306
1. Naked-eye appearances of the semen 306
2. Microscopical examination of the semen 306
3. Chemical examination of the semen 307

II. Secretions of the Sexual Organs of the Female . . 308
1. Mammary secretion (the milk) 308
2. Vaginal secretion 309
3. The uterine secretions 310
1. Menstruation 310
2. The lochia 310

CHAPTER X.

METHODS OF BACTERIOLOGICAL RESEARCH.

I. The Microscope 312

II. The Detection of Micro-Organisms 314

III. Cultivation of Micro-Organisms 316

A. Methods of Sterilisation 316
Sterilisation of instruments, fluids, and nutrient substances 316

PAGE

B. Nutrient Substances 318
1. Nutrient fluids 319
2. Solid nutrient substances 319
1. Blood serum 319
2. R. Koch's nutrient gelatine 319
3. Agar-agar 320
4. Potato 320
Stained nutrient substances 321

C. Preparation of Koch's Pure Cultivations 321
1. Plate cultivations 322
2. Cultivation by deep inoculation 324
3. Cultivations on glass slides 325
4. Cultivations in hanging drops 325

IV. The Transmission of Pure Cultivations to Animals . . . 325
(*a.*) By the lungs 325
(*b.*) In the food 325
(*c.*) By cutaneous inoculation 326
(*d.*) By subcutaneous inoculation and injection . . . 326

V. Scheme of a Bacteriological Investigation 326

APPENDIX.

Reaction of blood 329
Method of estimating the specific gravity of blood 329
The blood-plates 329
Bizzozero's chromo-cytometer 330
Amount of hæmoglobin 335
"Granules" in colourless blood-corpuscles 335
Stokes' fluid 336
Claude Bernard's method of estimating grape-sugar in blood . . 336
Griess' papers 337
Staining of tubercle bacilli in sputum 337
Triple phosphate 338
Test for hydrochloric acid 338
Proteids formed during gastric digestion 339
Dipteral larvæ passed alive from the bowel of a man 339
Gases in the stomach and intestine 339
Colour-scale for urine-testing 340
Reaction of urine 341
Albuminuria 342
Proteids in urine 342
Detection of serum-globulin in urine 343
Estimation of grape-sugar in urine by fermentation 343
The urine in febrile states 343
Chylous ascites 344
Abbe's condenser and apochromatic lenses 344

Bibliography 348
Index 380

LIST OF ILLUSTRATIONS.

FIG.		PAGE
1.	Blood-plates from normal blood. (The blood had been fixed with Hayem's solution, eye-piece III., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion)	6
2.	Capillary tube of *Thoma-Zeiss* apparatus for counting blood-corpuscles	8
3, 4, and 5.	*Thoma-Zeiss* counting-cell	9
6.	*Gowers'* hæmacytometer	12
7.	*Von Fleischl's* hæmometer	14
8.	Section of hæmatoscope	15
9.	*Hénocque's* instrument filled with blood	15
10.	Enamelled plate of the hæmatoscope	16
11.	Leukæmic blood, from a case of lymphatico-splenic leukæmia (eye-piece III., objective 8 A, *Reichert*)	20
12.	Eosinophile cells from leukæmic blood (eye-piece III., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror)	21
13.	Melanæmic blood (eye-piece III., objective 8 A, *Reichert*), from a case of malarial cachexia	23
14.	Poikilocytosis, from a case of amyloid degeneration of the kidney, liver, spleen, and intestine (eye-piece III., objective 8 A, *Reichert*)	24
15.	Anthrax bacilli, from rabbit's blood; from a preparation by *Professor Weichselbaum* (eye-piece III., objective *Reichert* $\frac{1}{15}$, homogeneous immersion; *Abbe's* mirror and open condenser)	29
16.	Anthrax bacilli, from human blood (*post-mortem*); from a preparation by *Professor Eppinger* (eye-piece III., *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror; open condenser)	30
17.	Spirillum of relapsing fever, from a photograph by *Koch*	31
18.	Tubercle bacillus in human blood, from a preparation by *Professor Weichselbaum* (eye-piece III., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror; open condenser)	32
19.	Bacillus of glanders, from a preparation by *Dr. Kolisko* (eye-piece V., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror; open condenser)	33
20.	Distoma hæmatobium, male and female, with eggs, from a preparation by *Dr. Schiess-Bey*	36
21.	Filaria sanguinis hominis, after *Lewis* and *Leuckart*	37
22.	Spectrum of oxyhæmoglobin	38
23.	Spectrum of gas-free reduced hæmoglobin	38
24.	Spectrum of hæmatin in alkaline solution	38
25.	Spectrum of reduced hæmatin	39
26.	*Teichmann's* hæmin crystals (eye-piece III., objective 8 A, *Reichert*)	39
27.	Spectrum of methæmoglobin in acid and neutral solutions	40
28.	Spectrum of carbonic oxide hæmoglobin	41
29.	*Hering's* spectroscope	44
30.	Micro-organisms of the buccal secretion, prepared by *Friedländer's* and *Günther's* methods (eye-piece III., objective *Reichert* $\frac{1}{15}$, homogeneous immersion; *Abbe's* mirror; open condenser)	54
31.	Thrush fungus from the mouth, from a patient with weak heart (eye-piece III., objective 8 A, *Reichert*)	58
32.	Leptothrix buccalis, from surface of tongue. The preparation was stained with iodo-potassic-iodide solution (eye-piece III., objective 8 A, *Reichert*)	59

FIG. PAGE

33. Nasal mucus (eye-piece III., objective 8 A, *Reichert*) 63
34. Epithelium, leucocytes, and crystals of the sputum (eye-piece III., objective 8 A, *Reichert*) 67
35. Elastic fibres in the sputum (eye-piece III., objective 8 A, *Reichert*) . 69
36. Spirals from sputum, in a case of bronchial asthma (actual size) . . 70
37. Spirals from sputum (eye-piece III., objective IV., *Reichert*) . . . 71
38. Fibrinous coagula, two-thirds natural size, from a case of pneumonia with paroxysms of severe dyspnœa 71
39. Fibrinous coagula, from a case of plastic bronchitis (two-thirds actual size) 72
40, 41. Moulds from the sputum in pulmonary abscess (eye-piece I., objective 8 A, *Reichert*) 73
42. Tubercle bacilli from sputum (eye-piece V., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror; open condenser) 77
43. Tubercle bacilli, stained by the *Ziehl-Neelsen* method (eye-piece III., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; open condenser) . . 78
44. Pneumonococci (eye-piece III., objective $\frac{1}{15}$ *Reichert*, homogeneous immersion; *Abbe's* condenser, without diaphragm) 79
45. Echinococcus hooklets and membrane of hydatid cyst (eye-piece III., objective 8 A, *Reichert*) 80
46. *Charcot-Leyden* crystals, from the sputum of an asthmatic patient (eye-piece III., objective VII., *Hartnack*) 81
47. Specimen of the vomit (eye-piece III., objective 8 A, *Reichert*) . . . 107
48. Specimen of the fæces (eye-piece III., objective 8 A, *Reichert*) . . . 128
49. Degenerated intestinal epithelium (eye-piece II., objective 8 A, *Reichert*) 129
50. Bacilli from fæces resembling bacillus subtilis (stained blue with iodo-potassic-iodide; eye-piece III., objective 8 A, *Reichert*) 133
51. *Nothnagel's* clostridia and stunted bacilli, which stain blue with iodo-potassic-iodide (from the fæces), (eye-piece III., objective 8 A, *Reichert*) 133
52. *Koch's* comma-bacillus, pure cultivation (eye-piece III., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror; open condenser) . . 134
53. *Finkler-Prior* bacillus, pure cultivation (eye-piece III., objective *Zeiss* $\frac{1}{12}$, homogeneous immersion; *Abbe's* mirror; open condenser) . . 137
54. Bacillus of typhoid (eye-piece III., homogeneous immersion objective *Zeiss* $\frac{1}{12}$). From a preparation by *Dr. Paltauf* 138
55. Animal parasites 140
56. Cercomonas intestinalis 141
57. Tænia saginata, head and proglottis (*Reichert's* eye-piece III., objective IV.) 143
58. Tænia solium, head (magnified), proglottis (actual size), and egg (magnified) 143
59. Tænia nana, head, proglottis, and egg 144
60. Tænia cucumerina, head and proglottis (magnified) 145
61. Bothriocephalus latus, head, eggs, and proglottides (eye-piece III., objective IV., *Reichert*) 146
62. Distoma hepaticum 147
63. Distoma lanceolatum (eight times actual size) and eggs 148
64. Ascaris lumbricoides: *a.* half natural size; *b.* slightly magnified; *c.* (eye-piece I., objective 8 A, *Reichert*) 149
65. Ascaris mystax, parasite and eggs 149
66. Oxyuris vermicularis and egg 150
67. Anchylostoma duodenale; *a, b.* actual size, *c, d.* slightly magnified, *e.* (eye-piece II., objective C, *Zeiss*) 151
68. Trichocephalus dispar: *a, b.* slightly magnified; *c.* (eye-piece II., objective 8 A, *Reichert*) 152
69. Trichina spiralis: *a.* and *b.* trichina of gut, slightly magnified; *c.* trichina of muscle (eye-piece III., objective IV., *Reichert*) 153
70. Anguillula stercoralis (head seen with eye-piece II., objective 8 A, *Reichert*) 154
71. Hæmatoidin crystals from acholic stools (eye-piece III., objective 8 A, *Reichert*) 155
72. Acholic stools (eye-piece III., objective $\frac{1}{15}$, oil immersion, *Reichert; Abbe's* mirror; narrow diaphragm) 156
73. Sulphide of bismuth crystals from the stools (eye-piece III., objective 8 A, *Reichert*) 158

FIG. PAGE

74. Urinary epithelium (eye-piece III., objective 8 A, *Reichert*), collected from thirty different specimens 178
75. Cast of urates, from a case of emphysema (eye-piece III., objective 8 A, *Reichert*) 181
76. Epithelium cast, from a case of chronic nephritis: *a.* fully differentiated; *b.* in part granular (eye-piece III., objective 8 A, *Reichert*) . . . 182
77. Cast of shrunken blood-corpuscles, in part metamorphosed (acute nephritis), (eye-piece III., objective 8 A, *Reichert*) 182
78. Cast formed of leucocytes, from a case of acute nephritis (eye-piece III., objective 8 A, *Reichert*) 182
79. *a.* and *b.* Rare forms of casts composed of leucocytes and epithelium; from a case of chronic nephritis which began with oliguria and uræmic paroxysms (eye-piece III., objective 8 A, *Reichert*) 182
80. *a.* and *b.* Granular cast in chronic nephritis (eye-piece III., objective 8 A, *Reichert*) 183
81. *a.* and *b.* Granular cast in acute nephritis (eye-piece III., objective 8 A, *Reichert*) 183
82. *a.* and *b.* Granular cast in chronic nephritis (eye-piece III., objective 8 A, *Reichert*) 183
83. Different forms of waxy casts: *a.* with a coating of urates; *b.* waxy casts covered with crystals of oxalate of lime; *c.* fragments of waxy casts (eye-piece III., objective 8 A, *Reichert*) 184
84. *a.* Granular cast beset with fatty globules and fat crystals; *b.* granular cast covered with leucocytes; *c.* and *d.* fatty casts; from a case of nephritis and large white kidney (eye-piece III., objective F, *Zeiss*) 185
85. Hyaline casts: *a.* hyaline cast; *b.* hyaline cast coated with leucocytes; *c.* hyaline cast covered with kidney epithelium; from a case of chronic hypertrophic hepatitis with jaundice (eye-piece III., objective 8 A, *Reichert*) 186
86. *a.* and *b.* Cylindroids from the urine in congested kidney (eye-piece II., objective 8 A, *Reichert*) 187
87. Micrococcus ureæ from the surface of a normal urine undergoing ammoniacal fermentation (eye-piece III., objective 8 A, *Reichert*) 189
88. Sediment from fermenting diabetic urine with casts of micrococci: *a, b, c.* various forms of urates; *d.* micrococci in form of casts; *e.* moulds; *f.* spore-fungi of bacilli and micrococci (eye-piece III., objective 8 A, *Reichert*) 190
89. Tubercle bacilli from urinary sediment in a case of tuberculosis of urinary organs (eye-piece III., objective $\frac{1}{15}$, oil immersion, *Reichert*) . . 192
90. Distoma hæmatobium in sediment (eye-piece II., objective C, *Zeiss*) . 193
91. Uric acid crystals from the urine in congestion of heart-disease (eye-piece III., objective 8 A, *Reichert*) 195
92. Pointed crystals of uric acid from the simple urine in congestion from emphysema (eye-piece III., objective 8 A, *Reichert*) 195
93. Oxalate of lime from sediment in a case of cystitis and pyelo-nephritis (eye-piece III., objective 8 A, *Reichert*) 196
94. Triple phosphate crystals from the sediment in chlorosis (eye-piece III., objective 8 A, *Reichert*) 197
95. Basic phosphate of magnesia (eye-piece II., objective C, *Zeiss*) . . 197
96. Neutral phosphate of lime from the urine of chronic nephritis after standing for twenty-four hours. The urine was feebly acid (eye-piece III., objective 8 A, *Reichert*) 197
97. Calcium sulphate, abundant sediment (eye-piece II., objective C, *Zeiss*) . 198
98. Hippuric acid (eye-piece II., objective C, *Zeiss*) 198
99. Hippuric acid from the urine of a rheumatic patient after large doses of benzoic acid had been taken (eye-piece II., objective 8 A, *Reichert*) . 198
100. (*a.*) Tyrosin; (*b.*) cystin; (*c.*) leucin (eye-piece II., objective 8 A, *Reichert*) 199
101. Lime and magnesia soaps from the urinary sediment of a woman with puerperal septicæmia (eye-piece II., objective 8 A, *Reichert*) . . 201
102. Triple phosphate crystals (rare form) from urine in alkaline fermentation (eye-piece II., objective 8 A, *Reichert*) 20

FIG. PAGE

103. Indigo crystals from a urine rich in indican, after standing for eight days at ordinary temperature (eye-piece III., objective F, *Zeiss*) . . . 203
104. Crystals of urate of ammonia, sediment in alkaline fermentation (eye-piece II., objective 8 A, *Reichert*) 203
105. Deposit of carbonates, sediment of ammoniacal urine (eye-piece II., objective C, *Zeiss*) 204
106. Cholesterin crystals, from the sediment in a case of tabes and cystitis; crystallised from æther and alcohol (eye-piece II., objective 8 A, *Reichert*) 204
107. *Esbach's* albuminimeter, one-half actual size 216
108. Phenyl-glucosazon crystals from diabetic urine (eye-piece II., objective 8 A, *Reichert*) 227
109. Fermentation flask for estimation of sugar, one-fourth actual size . . 233
110. *Lippich's* polarimeter, one-tenth actual size 234
111. Spectrum of urobilin in acid solution 241
112. Spectrum of urobilin in alkaline solution 241
113. *Ludwig's* filter, actual size 255
114. *Häfner's* apparatus for estimation of urea 257
115. Gonococci from pus of gonorrhœa (eye-piece III., objective, oil immersion $\frac{1}{12}$, *Zeiss*) 278
116. Gonococci from fresh pus 279
117. Cocci from an empyema, prepared by *Gram's* method (eye-piece III., objective *Zeiss* oil immersion $\frac{1}{15}$; *Abbe's* mirror and open condenser) . 292
118. Actinomyces granules in glycerine from actinomycosis of pleural cavity (eye-piece II., objective IV., *Hartnack*) 293
119. Actinomyces from a case of actinomycosis of pleural cavity (eye-piece III., objective, oil immersion $\frac{1}{15}$, *Reichert*) 294
120. Actinomyces from peritoneum (eye-piece III., objective, oil immersion $\frac{1}{15}$, *Reichert*) 294
121. Preparation from same case as last, stained by *Gram's* method (eye-piece IV., objective, oil immersion $\frac{1}{12}$, *Zeiss*; *Abbe's* mirror and open condenser) 295
122. Pus from putrid empyema (eye-piece III., objective 8 A, *Reichert*) . . 300
123. Contents of an ovarian cyst (eye-piece III., objective, 8 A, *Reichert*) . 303
124. Microscopical appearance of the semen (human) (eye-piece III., objective 8 A, *Reichert*) 307
125. Colostrum of a woman in sixth month of pregnancy (eye-piece III., objective 8 A, *Reichert*) 308
126. Preparation of vaginal secretion in a case of cancer of the cervix uteri (eye-piece III., objective 8 A, *Reichert*) 309

APPENDIX.

1. General view of *Bizzozero's* chromo-cytometer 332
2. Showing how the tubes fit into each other 332
3, 4. Mode of using as a chromometer 333
5. Microscope, *Reichert's* stand, No. II*b*., with draw-tube, rack-and-pinion fine adjustment, and *Abbe's* condenser 345
6. *Abbe's* condenser as made by *Zeiss*, side view 346
7. Same, viewed from above 347

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CHAPTER I.

THE BLOOD.

Every change in the quantity or the quality of the **blood** itself is apt to be attended with serious disturbance of the system; further, the blood is the carrier and distributer through the body of nearly all the poisons, organic and inorganic, which act upon the latter. From this it follows that the physiology and pathology of the blood represent a mass of knowledge, at once immense and various. It is not our purpose here, however, to treat these subjects exhaustively, but merely to select from them certain well-established facts which bear upon disease, and to point out the way in which they may help in its diagnosis.

I. COLOUR.—Arterial and venous blood differ considerably as to colour in health, the former being scarlet, and the latter a bluish red. The distinction, however, belongs not to the fluid part of the blood, or plasma, but to the colouring-matter, or hæmoglobin, contained in the red corpuscles, and it depends upon the chemical constitution of the corpuscles, which, changing colour themselves, determine the tint of the whole mass of the blood.

Thus, when the blood is rich in oxygen, the amount of hæmoglobin is increased, and the fluid is proportionately bright red. Again, where, as is always the case with venous blood, oxygen is deficient, or where, from physiological or pathological causes, arterial blood contains but little oxyhæmoglobin, the colour is darker, and this in a degree corresponding to the condition which underlies it. In certain morbid states, however, the blood may assume a brighter tint than that of healthy arterial blood. In carbonic oxide poisoning, for instance, it is of a bright cherry colour (see p. 41). [Venous blood, which appears dark red by reflected light, is green by transmitted light. It is therefore said to be dichroic. Arterial blood is monochroic.[1]]*

* The Numbers refer to the Bibliography at the end of the volume.

II. THE REACTION of healthy blood, like that of nearly all the tissue fluids, is alkaline. [It is due to the presence of disodic phosphate, Na_2HPO_4, and bicarbonate of soda.] Still, this reaction is liable to considerable variation under certain conditions of health as well as in disease.

The alkalinity of the blood diminishes when it has been withdrawn from the influence of living blood-vessels. Hence we find that an acid reaction is one of the phenomena of coagulation, and that this becomes more pronounced the longer the blood is allowed to stand. [The change depends upon the formation of an acid derived, probably, from the decomposition of the colouring-matter of the red corpuscles.]

To test the reaction of the blood, *Liebreich*[2] employs plates of plaster of Paris or clay soaked in neutral litmus solution. A few drops of the blood to be examined are placed upon this, and washed off again with water.* If the blood was alkaline, the spot upon which it has fallen exhibits a blue, but if acid, a red colour.

For the same purpose *Zuntz*[3] uses glazed litmus paper soaked in a solution of common salt or sulphate of soda; this he dips several times in the blood to be tested, and again washes in the salt solution. The same thing may be done by allowing a drop of the blood to fall on the litmus paper, and then quickly washing it off again, as in Liebreich's method. (See Appendix I.)

For the comparative estimation of the alkalinity of the blood in animals, *Lassar*[4] has devised a process, which, however, cannot be applied to the case of human beings, where the requisite quantity of blood is not available. On the other hand, the plan which *Landois*[5] recommends is very suitable for clinical purposes.

The author has obtained useful results in a large series of observations by proceeding according to the following method (a modification of that of Landois), for the quantitative estimation of the alkalinity of the blood :—

A mixture is made of a concentrated solution of sulphate of soda with 1/100 and 1/1000 normal solution of tartaric acid (I.) in various proportions; and in this way a series of test-fluids is obtained, the members of which contain varying quantities of acid to the cubic centimetre.†

* [The reaction of the blood with ordinary litmus paper is obscured by the red colour of the fluid, and the various expedients for its determination are directed to overcome this difficulty.]

† The fluids are prepared thus : 7.5 grms. of pure tartaric acid are dissolved in a litre of water, and a normal solution (1/10 of the acid) results. By appropriate dilution of this, the other normal solutions (1/100 and 1/1000) may be obtained. (*v. Jaksch*, Zeitschr. für klin. Medicin, xiii. 350, 1887.)

Experiment shows that eighteen such test-solutions of varying acidity are needed. And of these :—

I.	contains in 1 cc.	0.9 cc. of 1/100	normal solution of acid and		0.1 cc.	concentrated solution sulphate of soda.
II.	,,	in 1 cc. 0.8 cc. of 1/100	,,	,,	0.2 ,,	
		and so on.				
IX.	,,	in 1 cc. 0.1 cc. of 1/100	,,	,,	0.9 ,,	
X.	,,	in 1 cc. 0.9 cc. of 1/1000	,,	,,	0.1 ,,	
		and so on.				
XIV.	,,	in 1 cc. 0.5 cc. of 1/1000	,,	,,	0.5 ,,	
		and so on.				
XVIII.	,,	in 1 cc. 0.1 cc. of 1/1000	,,	,,	0.9 ,,	

The experiment is conducted in the following manner :—The proper quantities of the acid and sulphate of soda solutions are placed in a series of watch-glasses, by means of a pipette graduated in 0.1 cc.,* and a number of strips of very sensitive blue and red litmus paper are prepared. The blood is usually taken by means of cupping-glasses from the patient's back, and before it coagulates, 0.1 cc. of the blood is added to each cc. of the fluids described above, well mixed in each case, and the resulting mixtures tested with the litmus papers until one is found to exhibit a neutral reaction, *i.e.*, leaving the red or blue litmus paper unchanged. This will show what quantity of the acid is required to neutralise 0.1 cc. of the blood in question. In order to arrive at a reliable result, it is necessary to proceed very quickly, and as a rule it may be laid down that not more than $1\frac{1}{2}$ minute should be allowed to elapse between the taking of the blood and the conclusion of the experiment, having regard to the rapid diminution of alkalinity after the blood is withdrawn from the living vessels.

For the sake of clearness the following example may be taken :—

In the case of a man who suffered from tuberculosis and tabes dorsalis, it was found that 0.4 cc. of 1/100 normal tartaric acid solution was required to neutralise 0.1 cc. of blood.

1 cc. of 1/100 normal solution of the acid corresponds to 0.0004 grm. NaOH.

0.1	,,	,,	,,	,,	,,	0.00004 ,,	,,
0.4	,,	,,	,,	,,	,,	0.00016 ,,	,,

The alkalinity of the blood may, therefore, be expressed by 0.00016 grm. NaOH for 0.1 cc., or .160 grm. NaOH for 100 cc.

It is true, as *H. Meyer*[6] has shown, that the results to be obtained in this way are open to error. This, indeed, we should expect, since the final reaction varies in each specimen with the colour of the blood and the quantity of CO_2 which it contains. The method has been described here, faulty and unsatisfactory as it is, because by means of it certain information has been obtained as to the character of the blood in disease.

* For this purpose also the automatic pipettes (see p. 13) containing 0.1 cc. will serve very well.

Haycraft and Williamson's Method.

[The method recently introduced by *Haycraft* and *Williamson*[7] is very suitable for clinical purposes, since by its means the alkalinity of the blood may be determined quantitatively from a single drop of the fluid.

A number of red litmus papers is prepared, containing varying quantities of oxalic or some other acid. One of these is such as is found by experiment barely to give a reaction with normal blood, and this is made the basis of a series of test-papers answering to different degrees of alkalinity. The strength of each is estimated by means of a solution of caustic potash of known concentration. The papers are glazed, and dipped for a second or two in liquid paraffin and then dried. In conducting the experiment, a drop of blood is drawn from the finger (previously well cleansed) and placed upon a paper of medium strength. There it is allowed to rest for ten seconds. Sufficient of the plasma has then soaked in. The blood is washed off, and the reaction, if any, is at once apparent. Should this be so, a paper containing more acid is employed; but if there be no reaction, a weaker one is taken. Suppose now it is found that the blood will give a reaction with the sixth and not with the seventh paper, the former is then taken as the expression of its alkalinity. But it is known that an $\frac{1}{x^n}$ solution of an alkali will give the same reaction, therefore the alkalinity of the blood will be $\frac{1}{x^n}$. "This is perhaps not absolutely true, for probably the blood plasma does not percolate so readily into the litmus paper as does a watery solution of an alkali. In this case, however, the error will be uniform," and will not vitiate the conclusion in a series of comparative investigations.*]

The author's experiments[8] have led him to the conclusion that the alkalinity of 100 cc. healthy human blood corresponds to 260–300 mgrm. of NaOH. *Canard*,[9] who adopts a similar method, gives the equivalent as 203–276 mgrm. NaOH; while *Mya* and *Tassinari*,[10] from experiments upon blood drawn from the veins, quote very much higher figures (516 mgrm.). The alkalinity of the blood is often diminished in fever. The author has invariably found it reduced considerably in uræmia, as well as in certain toxic states, as carbonic oxide poisoning. [It is also reduced in persistent vomiting.] In organic disease of the liver, leukæmia, pernicious anæmia, and diabetes, the author has found such a diminution as was capable of being expressed in figures, and in this (the condition of the blood in chlorosis alone excepted) he is borne out by the researches of *Gräber*.[11] *Cantani*[12] is of opinion that the blood in cholera may exhibit an acid reaction even during life.

III. SPECIFIC GRAVITY OF THE BLOOD.—The specific gravity of healthy human blood has been stated at 1.045–1.075 by *Landois*,[13] and 1.035–1.068 by *Lloyd Jones*.[14] It is usually lower in women than in men; and the last-mentioned authority has shown that it is highest

* [I am indebted to the courtesy of Dr. J. B. Haycraft for a personal communication on the subject discussed in the text. He informs me that he has already derived important clinical inferences from the application of his method. By means of it he has ascertained that the reaction of the blood in different conditions may vary as widely as that of the urine.—(Ed.)]

at birth, falls gradually during the first few years of life, and reaches its lowest point in man at 35–45 years of age. [It is diminished by hunger, in pregnancy, by the ingestion of solid or liquid food, or by gentle exercise.[15]]

To estimate the specific gravity of the blood, *Roy's method* may be adopted. This requires a series of test-tubes, holding a mixture of glycerine and water in different proportions, so that the sp. gr. of these shall range between 1.040 and 1.080. Some twenty test-tubes will be needed, and each should hold 30–40 cc.

The mixtures may best be prepared in the following manner:—Two graduated burettes are filled,—one with water and the other with glycerine,—and from each of these a certain quantity of glycerine and water, varying in a uniform manner, is poured into the above-mentioned test-tubes, the total quantity of the mixture being always 25 cc. Thus, in the first is placed 24 cc. water and 1 cc. glycerine; in the second, 23 cc. water and 2 cc. glycerine, and so on. In this way is obtained a series of fluids of uniformly varying sp. gr., as may be shown by the hydrometer. Into the fluid in each test-tube is now let fall a drop of blood, which will remain suspended in one of them, neither sinking nor rising; the specific gravity of this mixture is that of the blood. Very good approximate results may be obtained by this somewhat tedious process. [Instead of glycerine in this experiment, *Landois* employs solutions of sodic sulphate ranging between 1.050–1.070 sp. gr., and the blood drawn from the finger is taken up in a capillary tube and expressed beneath the surface of the fluid. (See Appendix II.)]

IV. CHANGES IN THE FORMED ELEMENTS OF THE BLOOD.—It is well known that the blood contains red and white corpuscles, and recent observations (*Bizzozero*) have shown the presence in it of a third class of formed elements,—the blood-tablets (or blood-plates). The existence of these bodies is now beyond dispute. To make them apparent in fresh blood, it is necessary to fix the latter by the addition of some preserving fluid, such as *Hayem's*[16] solution, when it may be examined directly with an oil-immersion lens and a narrow diaphragm. (See Appendix III.).

The constitution of *Hayem's solution* is as follows:—

1 grm. of chloride of sodium, 5 grms. of sodic sulphate, 0.5 grm. corrosive sublimate, and 200 grms. distilled water.

The preparation will then show the bodies in question as minute objects with a diameter less than half that of the red blood-corpuscles, scattered singly, or in groups in the field. In the present state of our knowledge, they possess no diagnostic importance. [They are sup-

posed to be most abundantly present in the blood of persons suffering from chronic diseases; and are said to increase in number during pregnancy (*Halla*), in conditions of regeneration (*Afanassiew*), in febrile anæmia (*Fusari*), and to diminish in fever.[17]]

The physiology of the red and white blood-corpuscles is sufficiently set forth in the Text-books of that science.[18]

Pathologically, the corpuscles exhibit changes as to quantity and character which are of the utmost importance in diagnosis. These changes seldom occur separately, but are usually combined,—although alterations of the *structure* of the corpuscles may be more pronounced in some cases, of their *number* in others. We shall consider :—

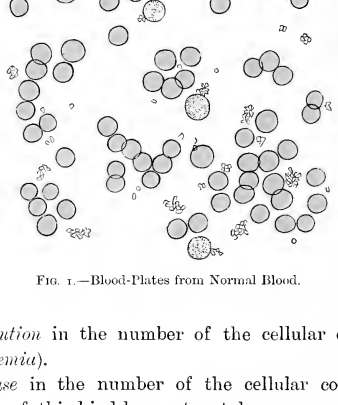


Fig. 1.—Blood-Plates from Normal Blood.

1. The *diminution* in the number of the cellular elements of the blood (*oligocythæmia*).

2. The *increase* in the number of the cellular constituents. An absolute increase of this kind has not yet been proved to occur, but a relative preponderance of white corpuscles is often met with. This happens normally during digestion (*physiological leucocytosis*),—as a transient phenomenon in a number of morbid states (*pathological leucocytosis*), and as a persistent condition (*leukæmia*).

3. Changes in the form of the blood-corpuscles (*poikilocytosis*, *mikrocythæmia*).

1. Oligocythæmia.—*Vierordt* has computed that in health the number of red blood-corpuscles is five millions in a man, and $4\frac{1}{2}$ millions in a woman to the cubic millimetre of blood.[19] In disease the number may diminish temporarily or permanently to two millions, or even sink as low as 360,000 per cubic millimetre. Such a condi-

tion may occur as a consequence of hæmorrhage, whether of a traumatic origin, or due to morbid changes in the blood-vessels, as when intestinal bleeding takes place in typhoid fever. As a permanent state, it may be a phenomenon of any disease which is attended with deficient regeneration of the blood.

[The number of the red corpuscles is lessened in chronic lead-poisoning, miasmatic conditions, and in syphilis.]

Diagnosis of Oligocythæmia.—The methods and apparatus employed by physiologists to estimate a diminution of the red blood-corpuscles are very many; but of these a large number are useless for clinical purposes, inasmuch as they require too great quantities of blood to work upon.

The apparatus which will serve our purpose is of two classes. One is used to count the actual number of blood-corpuscles in a specimen of blood; the other, by estimating the quantity of hæmoglobin present, enables us to draw an inference as to changes in the blood. Both methods have their advantages and supplement each other, since a diminution in the hæmoglobin is usually proportionate to a diminution in the red corpuscles; thus, *oligochromæmia* and *oligocythæmia* mostly occur together.

When the *oligocythæmia* is very pronounced, a glance through the microscope will suffice to recognise it; and so with *oligochromæmia*—diminution of hæmoglobin—a little practice will enable us to detect it by a simple examination of the blood in a very thin layer without the addition of any fluid. To effect this, the end of the finger should be washed in plain water, and pricked, and the first drops of blood allowed to flow off. A glass slide should then be allowed to touch the summit of the drop of blood on the skin without coming in contact with the finger, quickly withdrawn, and a cover-glass placed over it.

In this way such impurities as epithelium, &c., are avoided.

The use of carbolic acid, æther, or alcohol, to wash the skin, is not to be recommended, since these bodies are likely to produce changes in the appearance of the corpuscles. When the object of the examination is the detection of micro-organisms in the blood, the utmost care must be taken in cleansing the skin (see p. 27).

Proceeding in this way in a case of oligocythæmia, when the slide is placed under the microscope, a marked diminution in the number of blood-corpuscles will be noticed. The red corpuscles will also in most cases be paler than normal; their usual bi-concave shape less marked; they are somewhat flattened, and they tend less to run into rouleaux or assume stellate forms. At the same time, they may be seen to have undergone peculiar changes of shape (*poikilocytosis*).

For many purposes it is well to fix the blood with some preserving

fluid before examination. A solution of common salt (0.8–1.0 per cent.) or of sulphate of magnesium (5 per cent.) may be used. *Grüber's*, *Hayem's*, or *Pacini's* solution may be employed. Hayem's has been already described.

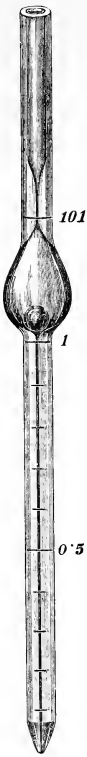


FIG. 2.—Capillary Tube (Thoma-Zeiss Apparatus for Counting the Blood Corpuscles).

Pacini's solution is prepared thus :—A mixture is made of 1 part corrosive sublimate, 2 parts common salt, 13 parts glycerine, and 113 parts distilled water, and the fluid is allowed to stand for at least two months. When about to be used, a portion is diluted with three times its bulk of distilled water, and filtered through blotting-paper.[20]

When it is a question of a lesser degree of oligocythæmia, this proceeding will not suffice, and we must have resort to special means of estimating the precise number of the corpuscles or the relative quantity of hæmoglobin present. In recent times a great many instruments have been constructed with the first-named object—as those of *Quincke*, *Malassez*, *Hayem*, *Thoma-Zeiss*, and *Gowers*. The principle on which all these are constructed is the same. A known quantity of blood is mixed in definite proportion with some indifferent fluid (3 per cent. salt solution, &c.), a portion of the mixture is placed upon a hollow slide of known contents and graduated surface, and then the corpuscles are counted with the aid of the microscope.

(*a.*) **The Thoma-Zeiss Apparatus for Counting Blood-Corpuscles.**—The simplest and best of these instruments is that of Thoma and Zeiss. It consists of a capillary tube of glass about 10 centimetres long, expanding in its upper third to a bulb, in which lies a small glass ball. The lower end of the tube is furnished with a scale, graduated in parts, numbered 0.1, 0.5, 1, up to 101 (fig. 2). With this instrument is used a counting-chamber invented by *Abbe*[21] and *Zeiss*. This is a glass receptacle cemented upon a glass slide (fig. 3); it is exactly 0.1 mm. in depth, and its floor is marked out into microscopic squares (fig. 4). The space overlying each square = 1/4000 mm^3 * and the squares are portioned out in groups of 16 by plainer lines (fig. 5).

Application of the Process.—A puncture is made in the tip of the finger, and in doing this the precautions already indicated are taken.

* Mm^3 = cubic millimetre.

Blood from the summit of the exuding drop is then sucked into the tube until it reaches the mark 0.5 or 1. The point of the tube is wiped, and a 3 per cent. solution of common salt sucked in until the fluid has risen to the point marked 101. The contents of the tube are then thoroughly mixed, and the column of fluid in the capillary tube is removed by blowing into the tube, as the blood would not mix with the solution of common salt. To neglect this precaution, therefore, would vitiate the experiment.

The capillary tube must be carefully cleaned after use, by washing it with distilled water, then with alcohol, and finally with æther, and a brisk current of air blown through it. For the latter purpose *Böhm's* air-pump is very suitable.

Fig. 3.

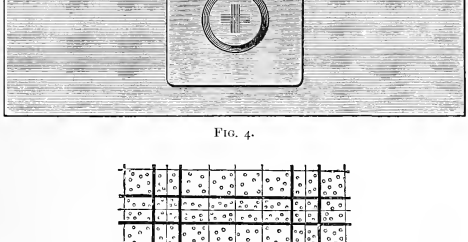


Fig. 4.

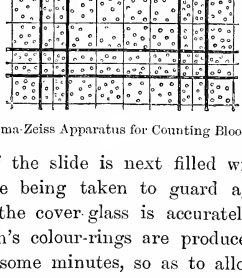


Fig. 5.—Thoma-Zeiss Apparatus for Counting Blood-Corpuscles.

The hollow cell of the slide is next filled with the mixed blood-and-salt-solution, care being taken to guard against the admission of air-bubbles, and the cover-glass is accurately adjusted in such a manner that Newton's colour-rings are produced. The preparation is left to stand for some minutes, so as to allow of an intimate admixture of its parts, after which it is placed under the microscope, and looked at with a power of 30–70 diameters, when it will be seen whether any air-bubbles or foreign bodies are present in it, and

whether the corpuscles are pretty evenly distributed through the fluid. The latter are then counted under a high power. In doing this, the number present in sixteen squares is counted, and from this the average is estimated. The greater the number of the squares taken, the more accurate will be the result attained. To count the corpuscles contained in sixteen squares, *Lyon* and *Thoma*[22] make the following suggestions:—

The space overlying a vertical series of four of the squares is taken as that the contents of which are to be estimated together. All the cells which cover or impinge upon the upper boundary of the rectangle formed by these four squares are to be reckoned, and that whether the cells themselves are situated within or without the boundary-line in question. So also are those which touch upon the line bounding the field of four squares on one (left) of its sides, and all that are entirely included within its limits without being anywhere in contact with the boundary.

The object-glass used in this investigation should be *Zeiss*, C or D; *Hartnack*, 6; or *Reichert*, 7.

The estimation of the total number of corpuscles is conducted as follows:—If the blood in the tube reached to the point 0.5, its proportion in the mixed solution will be 1 : 200; if to the point 1.0, 1 : 100. Multiply the number of corpuscles counted in all the squares by 4000 ($\frac{1}{4000}$ being the cubic contents overlying a square), and the result by 100 or 200, according to the degree of dilution. Then divide the product by the number of squares taken, and the result gives the number of blood-corpuscles contained in a cubic millimetre of blood.

To estimate the number of *white* corpuscles in a specimen of blood, Thoma dilutes the latter with water containing one-third per cent. glacial acetic acid in the proportion of 1 : 10. In this way the red corpuscles are destroyed, and the white alone remain in the field of vision. In mixing the fluids, the same observer employs a mixing-glass specially devised by Zeiss for the purpose. The process may also be carried out thus:—By means of a pipette of 1 cc. contents, and accurately graduated in 0.1 cc. units, 0.9 cc. of the acetic acid solution is measured out into a watch-glass; with another pipette holding exactly 0.1 cc. the blood is added to this, and the two well mixed. A drop of the mixture is placed within the counting-chamber of the cytometer prepared as before. And now, since the number of corpuscles is relatively fewer, the entire field, and not its marked-out divisions, is taken as the basis of calculation, greater accuracy being so obtained. To the same end, a lower power will be used, so as just to bring the marks on the floor of the chamber clearly into view. Before beginning to count the corpuscles, however, it will be well to focus with the fine adjustment of the microscope, and to make sure that the cells have all settled.

The cubic contents of that part of the chamber which corresponds to the field of vision can be ascertained in the following way:—The divisions of the chamber which appear in the field are first counted. Each of these measures $\frac{1}{20}$ mm. across (v. *supra:* the area $= \frac{1}{400}$ mm^2, the cubic contents $= \frac{1}{4000}$ mm^3). The diameter of the field, therefore, is $\frac{1}{20}$ mm. multiplied by the number of divisions which it contains. Thus, for instance, if ten of the divisions are seen, the diameter of the field $= 10 \times \frac{1}{20}$ mm. or $\frac{10}{20}$, and the radius $= \frac{10}{40}$ mm. The area of the field, therefore $= \pi \left(\frac{10}{40}\right)^2$ mm^2, and if the chamber is 0.100 mm. in depth, its cubic contents $= 0.1 \times \left(\frac{10}{40}\right)^2 \pi$ cubic mm. Hence we obtain the following formula: *—

$$\frac{10 \times Z}{M \times Q}$$

where M = the number of divisions under the microscope, Z = the number of cells counted, Q = the cubic contents of the field ($Q = 0.1\ \pi R^2$, where R = the radius of the field in mm.), and where the blood is diluted in the proportion of 1 : 10. The formula will give us the number of cells contained in a cubic millimetre of undiluted blood.

When there is a very great increase in the number of leucocytes, as in leukæmia, their number can be estimated in the same manner as that of the red corpuscles, and the relative proportion of the two can be determined at the same time with sufficient accuracy, if only an adequate number of squares is taken into account. Great assistance in such experiments may be derived from the use of a 3 per cent. salt solution coloured with gentian violet, in which the leucocytes are stained, and become readily discernible from the red blood-corpuscles, which are usually somewhat paler than normal. For the same purpose, *Mayet*[23] recommends that the blood be mixed with perosmic acid, glycerine, and water, which is said to confer upon the coloured blood-corpuscles a beautiful red tint, whilst the white corpuscles remain unaffected by it.

[(*h.*) **Gowers' Hæmacytometer.**†—This instrument is the most commonly used for clinical purposes in this country. "The hæmacytometer (fig. 6) consists of: (1.) A small pipette, which, when filled to the mark on its stem, holds exactly 995 cubic millimetres. It is furnished with an india-rubber tube and mouth-piece to facilitate filling and emptying. (2.) A capillary tube marked to contain exactly 5 cubic millimetres, with india-rubber tube for filling, &c. (3.) A small glass jar in which the dilution is made. (4.) A glass stirrer for mixing the blood and solution in the glass jar.‡ (5.) A brass stage-plate, carrying a glass slip, on which is a cell

* $\pi = 3.1416$. mm^2 = square millimetre. mm^3 = cubic millimetre.

† For the above description and figure we are indebted to *Landois and Stirling's* Physiology. p. 19.

[‡ In practice error is apt to arise from variation in the depth of the cell, and it is not easy to obtain one precisely $\frac{1}{5}$ mm. Assuming that the same instrument is always used by the observer, this is best corrected by ascertaining the true depth and allowing for the error in mixing the solution. Suppose, for instance, the cell is found to have a

$\frac{1}{5}$ of a millimetre deep. The bottom of this is divided into $\frac{1}{10}$ millimetre squares. Upon the top of the cell rests the cover-glass, which is kept in its place by the pressure of two springs proceeding from the ends of the stage-plate."

The method of employing the instrument is as follows:—The diluting solution used is a solution of sodic sulphate in distilled water, sp. gr. 1.025, or the following:—Sodic sulphate, 104 grains; acetic acid, 1 drachm; distilled water, 4 oz. "995 cubic millimetres of the solution are placed in the mixing jar; 5 cubic millimetres of blood are drawn into the capillary tube from the puncture in the finger and then blown into the solution. The two fluids are well mixed by rotating the stirrer between the thumb and finger, and a small drop of this dilution is placed in the centre of the cell, the covering glass gently put upon the cell, and secured by the two springs, and the plate placed upon the stage of the microscope. The lens is then focussed for the squares. In a few minutes the corpuscles have sunk

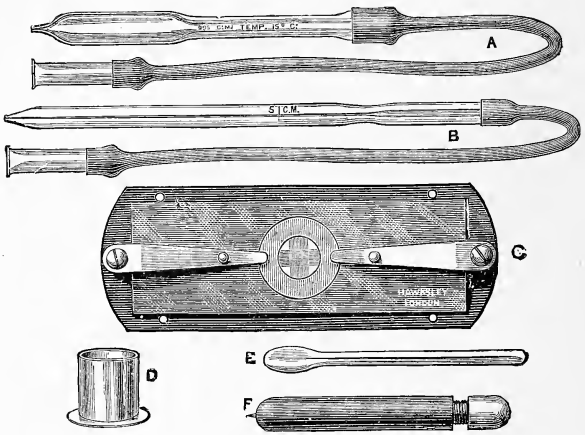


FIG 6.—Gowers' Apparatus. A, pipette for measuring the diluting solution; B, capillary tube for measuring the blood; C, cells, with divisions on the floor, mounted on a slide; D, vessel in which the dilution is made; E, glass stirrer; F, guarded spear-pointed needle.

to the bottom of the cell, and are seen at rest on the squares. The number in ten squares is then counted, and this, multiplied by 10,000, gives the number in a cubic millimetre of blood." [24]]

To estimate the COLOURLESS corpuscles only, mix the blood with ten parts of 0.5 per cent. solution of acetic acid, which destroys all the red corpuscles [*Thoma*].

The instruments of *Bizzozero*, *v. Fleischl*, *Hénocque*, and *Gowers* [25] involve the second principle to which allusion has been made, viz., the estimation of the quantity of hæmoglobin in the blood. (See Appendix IV.)

(*c.*) **Bizzozero's Chromo-cytometer.**—This instrument consists of two tubes, one within the other, both being closed at the same end by a

depth of 150 μ instead of 200 μ, 5 parts of blood should be added to 945 of the diluting fluid, instead of to 995. The results so obtained will be absolutely accurate (*Hart*).]

glass disc, while the other end remains open; connected with the first tube, and communicating with its cavity by means of a slit which reaches as far as the disc closing its lower end, is a small open reservoir. By moving the inner tube within the outer, the space between the glass discs which close them can be increased or diminished at will, and the thickness of the layer of fluid, which enters freely from the reservoir, can be made to vary.

To use the instrument as a **cytometer**, blood should be drawn as already directed, and diluted with a known proportion of common salt solution. The thickness of the column of fluid which will just allow the flame of a candle to be visible at $1\frac{1}{2}$ metre from the instrument is then ascertained.

When it is employed as a **chromometer**, the blood must be so mixed with a known quantity of water that the dilution of the hæmoglobin will allow the coloured fluid to be transparent. The proportion of contained hæmoglobin can then be estimated by measuring the thickness of the column when the fluid is of the same colour as a standard solution of oxyhæmoglobin, which accompanies the instrument.

The author has had no personal experience in the use of this instrument, but the experiments of Bizzozero and others lead him to expect that much may be learnt from it with reference to the amount of hæmoglobin in the blood.

(*d.*) **Von Fleischl's Hæmometer.***—The application of this instrument (fig. 7) depends upon the principle that the colour of the blood diluted with water may be compared with that of a glass wedge tinted with Cassius's golden-purple, or some such pigment.

Its essential part is the red glass wedge, which is mounted movably beneath a platform like that of a microscope, with a circular opening in its centre. Upon this the light from a gas or oil lamp (daylight is not admissible) is projected by a plate of plaster of Paris. Above the wedge, and exactly over the circular opening in the platform, is fixed a metallic tube $1\frac{1}{2}$ cm. long, closed at the bottom with a plate of glass and divided by a vertical metallic partition, so that one half of the metallic tube receives its light through the red glass wedge, the other directly from the white reflector. When the apparatus is in use, the former of these compartments is filled with pure water, the other with water mixed with a known quantity of blood.

To secure this known quantity, von Fleischl has designed an automatic blood pipette of such a capacity that, when healthy blood is used, the resulting mixture corresponds in colour to that derived from the part of the red glass wedge which is marked 100. From this point to its sharp edge (where 0 stands), the wedge is graduated in ten divisions,

* *Von Fleischl's* instrument is made by *Reichert* of Vienna, and sold for 35 florins.

which represent its diminishing thickness, the Nos. 90, 80, &c., being marked on the apparatus.

The instrument is employed thus:—The blood is obtained from a puncture in the finger, and placed by means of the pipette in the proper compartment of the tube. Both compartments are then filled with water, and the red glass wedge is moved until the two fluids show an equal intensity of red colour. The number indicated on the scale is then read off. Suppose this should be 80,—then the blood examined con-

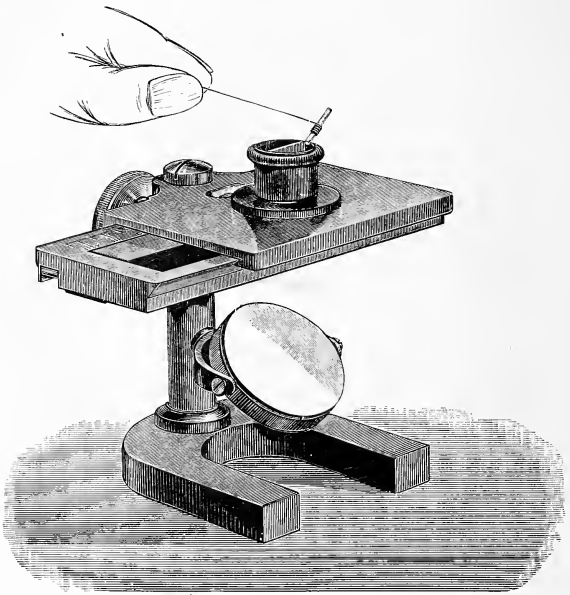


Fig. 7.—Von Fleischl's Hæmometer.

tains but 80 per cent. of the normal proportion of hæmoglobin, or the quantity of hæmoglobin is to that of healthy blood as 80 : 100. Now assuming that in a healthy man 14 grms.* may be taken as the amount of hæmoglobin in 100 grms. of blood, we can calculate the latter absolutely for the specimen examined by means of the formula:

$$X = \frac{14 \times R}{100}$$

* *J. G. Otto* states the normal quantity at 13.77 per cent. 14 is taken here (as by *Hénocque* elsewhere) as a simpler expression for the purposes of the calculation. (See Appendix V.)

Where
X = the quantity of hæmoglobin in 100 grms. of blood;
R = the figure obtained with von Fleischl's apparatus to express the relative proportion of hæmoglobin in the blood; and
14 = the normal quantity of hæmoglobin in the blood of a healthy adult.

Although it must be conceded that the results obtained with this instrument are not absolutely correct, it still supplies a simple and ready means of estimating the hæmoglobin in the blood, and has the further advantage that it needs but little of the latter to work upon. It has proved a useful adjunct to the *Thoma-Zeiss* apparatus, or that of Gowers, in the investigation of changes in the blood for clinical purposes.[26]

(*e.*) **Hénocque's Hæmatoscope.**[27]—This instrument has the advantage over others employed for a similar purpose, that while comparatively little fluid is needed for its application, this consists of pure blood undiluted with artificial serums.

It consists essentially of two glass plates superimposed in such a

FIG. 8.—Section of Hæmatoscope.

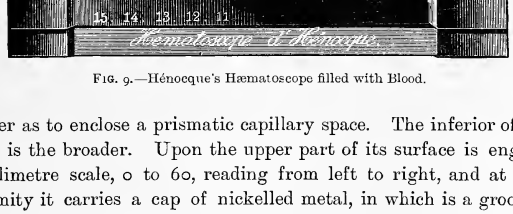


FIG. 9.—Hénocque's Hæmatoscope filled with Blood.

manner as to enclose a prismatic capillary space. The inferior of these plates is the broader. Upon the upper part of its surface is engraved a millimetre scale, 0 to 60, reading from left to right, and at either extremity it carries a cap of nickelled metal, in which is a groove for the reception of one end of the smaller (upper) plate. These grooves are so placed that whilst the plates are in immediate contact at one end, that opposite 0 of the scale, they are separated at the other by an interval of 0.3 mm. The smaller plate can be made to slide in the grooves under gentle pressure, and can thus be removed for the purpose of cleaning the instrument. From the above description it will be seen that a layer of blood introduced within the capillary chamber has a thickness varying uniformly from 0 on the left to 0.3 mm. under the

mark 60 on the graduated scale; and it also follows that its depth increases by 0.005 mm. for every mm. distance towards the right. Thus its depth at any point may be known by multiplying the corresponding figure on the scale by five, when the result is expressed in thousandths of a millimetre or micron.

The prismatic chamber is filled with blood obtained by pricking the finger, and this is done best by bringing the lower lamina on a level with the puncture and permitting the issuing drops to flow upon it at a gentle decline. The blood will then arrange itself in an even layer, and if its continuity be interrupted by empty spaces or air-bubbles, these can be extruded by slightly tapping the glass wall with the finger-nail. Six drops generally suffice. The edges of the instrument, when filled, are wiped clean, and the examination may be begun.

This is conducted in two different ways :—

a. The first (*procédé diaphanométrique*), the readier and simpler, has for its object to estimate the relative opacity of the blood, and so to infer the proportion of hæmoglobin which it contains.

For this purpose there is supplied with the hæmatoscope a plate of

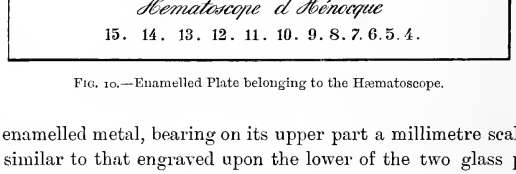


Fig. 10.—Enamelled Plate belonging to the Hæmatoscope.

white enamelled metal, bearing on its upper part a millimetre scale precisely similar to that engraved upon the lower of the two glass plates, and below a descending series of figures, of which the first underlies the 8 mm. mark above. Then follow at constantly diminishing intervals the figures 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4. The markings upon this plate are traced throughout in black. When in use, it is placed behind the hæmatoscope filled with blood, and held there in such a position that like markings upon the millimetre scale accurately correspond. It is evident that the portion of the blood layer which is thinner, and therefore less deeply coloured, will be transparent, and will suffer the marks beneath to be visible, while these disappear towards the thicker end. The examination consists in noting the point at which the figures (15–4) in the lower series cease to be visible; and it is clear that this point will be attained the sooner according as the blood is richer in hæmoglobin.

Further, Hénocque has arranged the series so that the second figure (14) expresses in grms. the quantity of oxyhæmoglobin in 100 grms. of blood, and this figure terminates the series as seen through a layer of blood of normal constitution. With blood taken from a case of anæmia, on the other hand, the figure 8 or 7 may be legible, and this implies that such blood contains in 100 grms. only 8 or 7 grms. of oxyhæmoglobin. Finally, the thickness of the stratum of blood at the point of requisite opacity may be ascertained from the mm. scale in the manner already indicated.*

(*β.*) In the second and more accurate mode of using the hæmatoscope the enamelled plate is dispensed with, and a Browning's spectroscope is required. The instrument, filled with blood as before, is placed opposite the cleft of the spectroscope, and the point is observed at which the characteristic spectrum of oxyhæmoglobin is first distinctly formed,—when the corresponding point on the millimetre scale of the glass plate is read off. The less hæmoglobin in the blood, the thicke must be the layer from which a spectrum is obtained. In order to secure a correct reading from the scale, it is well to place the apparatus holding a stratum of blood upon a sheet of white paper against a window so as to examine it by bright and diffused daylight, and then directing the spectroscope over 1 or 2 cm. of its surface, the observer should several times judge for himself concerning the point of earliest definition of the spectrum. Of the numbers obtained in this way (which will usually differ by an amount expressing only two or three millimetres) the mean is taken, and employed for the purpose of the calculation. It must be allowed that the conclusion in this respect is always somewhat arbitrary, and leaves room for a difference of opinion as to when precisely the spectrum is formed; but once the eye has become accustomed to look for a certain clearness in the outline of the bands, it seeks for and easily appreciates it in every instance.†

From the reading on the scale at the point where the spectrum is thus seen, the thickness of the blood stratum, and the quantity of oxyhæmoglobin in a known quantity of blood, can be readily determined. In the case of normal blood, which contains 14 grms. of oxyhæmoglobin in 100 grms. of the fluid, the absorption-bands are plainly visible in the situation of the figure 14 on the mm. scale; and from what

* [Hénocque's hæmatoscope may be obtained from M. Lutz, 82 Boulevard Saint-Germain, Paris. The price is 12 francs, and the enamelled plate costs 5 francs additional.—(Ed.)]

† [This difficulty may be further obviated by the use of Hénocque's double spectroscope, by means of which two persons are enabled to make the observation at once. For a description of this instrument the reader is referred to the original communication, "L'Hémato-spectroscope." Compt. Rend., Soc. de Biologie, October 1886.]

has been already said it follows that the thickness of the blood stratum at this point is 14×0.005 mm. $= 0.07$ mm. Let it be assumed now that in a given case the bands just become distinctly evident at a point corresponding to the division 20 on the index; then the thickness of the layer which yields them is $20 \times 0.005 = 0.1$ mm. From these data the quantity of oxyhæmoglobin in 100 grms. of the blood may be calculated by the following equation :—

$$x : 14 = 0.07 : 0.1$$

$$x = \frac{14.0.07}{0.1} = 9.8$$

that is, 100 grms. of the blood examined holds 9.8 grms. oxyhæmoglobin. In this formula :

x = the quantity of oxyhæmoglobin sought.

14 = the quantity of oxyhæmoglobin in 100 grms. of healthy blood.

0.07 = the thickness of the blood stratum, which will make the absorption-bands plainly evident where the blood, as in normal blood, holds 14 grms. oxyhæmoglobin in 100 grms. of the fluid.

0.1 = the thickness of the blood stratum which shows the bands in the example chosen.

To obviate the necessity for making the calculation afresh in each case, Hénocque has compiled a table from which the quantity of hæmoglobin may be deduced directly from the depth of the blood stratum.

Comparisons which the author has instituted between the results arrived at in this way with others derived by means of v. Fleischl's apparatus, have shown that the two are sufficiently in accord. *Henschen*,[28] however, is of opinion that the latter are more accurate, and accounts for this by pointing out that they are obtained from oxyhæmoglobin, whereas with Hénocque's hæmatoscope that body is still within the cells.

The preference will usually be given to *v. Fleischl's* apparatus, because of the greater quantity of blood needed for the application of Hénocque's hæmatoscope; but the latter is especially suitable for the observation of such changes in the blood as the formation of methæmoglobin, &c., which can be recognised by spectrum analysis. To detect such changes, Hénocque has applied it in a very ingenious manner. He observed the development of oxyhæmoglobin-bands in transparent parts, such as the lobe of the ear and the ungual phalanges of the finger, capable of being illuminated by diffuse sunlight.* Then, in the case of the ungual phalanx, the part was ligatured, and he noted the length of time required for the appearance of the broad absorption-band of reduced hæmoglobin. Proceeding in this way, he found that

* [In researches of this character the double spectroscope is specially recommended by *Hénocque*.]

with a normal proportion of oxyhæmoglobin, reduction took place in the course of 70 seconds, while with anæmic blood the interval was shortened to 30–40 seconds.

As a result of his researches, Hénocque has arrived at the following formula, which is applicable to clinical purposes:

$$E = \frac{M}{D} \times 5$$

Where

E = the energy of reduction;
M = the mean proportion of hæmoglobin ascertained by his method;
D = the time in seconds in which reduction is accomplished.[29]

The formula is derived from the following considerations:—In a specimen of blood holding 14 grms. oxyhæmoglobin in 100 grms., reduction takes place in 70 seconds, and in another holding 13 grms. in 100, it takes place in 65 seconds. Inspection of the figures shows that in both cases a fifth part of the quantity of oxyhæmoglobin (in 100 grms.) is reduced. Hence, to obtain the value of E (energy of reduction), the quantity of oxyhæmoglobin found is multiplied by 5, and the product divided by the number expressing the time (in seconds) in which reduction takes place. Experiments are now in progress under the author's supervision which have for their object to ascertain how far this method of investigation may be of use in diagnosis. The apparatus may be employed also for the examination of milk, in the spectrum-analysis of urine and morbid fluids, and for the aniline dyes of so much consequence in staining processes. It seems to merit a description here on account of its extended utility; and in any case, in connection with the spectroscopic examination of the blood, it must be classed with the apparatus of Thoma-Zeiss, Gowers, and v. Fleischl as a valuable addition to our resources.

2. Leucocytosis.—By this term is meant a condition in which the number of white corpuscles in the blood is temporarily much increased. Such an increase occurs in health as part of the process of digestion.[30] One to two hours after the principal meal, the white corpuscles are found in the blood in the proportion of 1 : 150 or even 1 : 100 of red corpuscles, diminishing soon after to 1 : 350 or 600, or, according to *Gräber*,[31] between 1 : 521 and 821. Much greater degrees of leucocytosis, which for the most part is transitory, are found in disease. *Virchow*[32] maintains that every affection in which the lymphatic glands are involved leads to this condition, and it is also present in many infectious diseases, such as relapsing fever and typhoid. According to *Tumas*,[33] leucocytosis occurs regularly in croupous pneumonia, a fact confirmed by the independent investigations of the author.

Leucocytosis can be readily diagnosed with a little practice by means of the microscope alone. For the more precise estimation of its degree the Thoma-Zeiss instrument may be used. It is necessary, above all things, that the blood examined should not be rich in digestion-products, and on this account it should not be taken until some hours after a meal. It is important to be able to recognise a pathological

leucocytosis. Its presence, taken in conjunction with the clinical symptoms, will often serve to clear up the diagnosis of diseases,—as, for instance, osteomyelitis,—which, without its light, would be very obscure.

3. Leukæmia.—This condition, when well marked, may be recognised from the naked-eye characters of the blood (*Virchow*),[34] which, when allowed to flow from the finger, is light red in colour, and somewhat turbid and greasy, as though loaded with fatty matter.[35]

The reaction of the blood is alkaline (*Mosler*[36]), not acid, as was formerly thought, but its alkalinity is often considerably less than normal (*v. Jaksch*). Microscopical examination shows an enormous increase in the number of white corpuscles [*J. H. Bennett*]. *Virchow* has found them in the proportion 2 : 3 of red corpuscles; *J. Vogl*, 1 : 3 to 1 : 2; *Schreiber*, 2 : 3.[37] In five cases recently treated in Nothnagel's clinic, the proportion was 1 : 3, 1 : 5, 1 : 8, 1 : 11, 1 : 12 (*Gottlieb*).

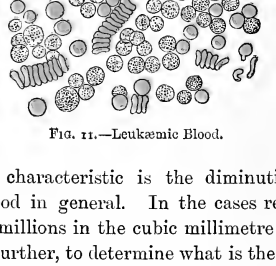


Fig. 11.—Leukæmic Blood.

Another notable characteristic is the diminution of the cellular elements of the blood in general. In the cases recorded above, their number fell to 2–3 millions in the cubic millimetre of blood.

It is important, further, to determine what is the prevalent character of the leucocytes present in the blood. By observing this, the author believes, in spite of *Bizzozero's*[38] assertion to the contrary, that it is possible to ascertain with what form of the disease we have to deal. It is usual to distinguish leukæmia as splenic, lymphatic, and myelogenic, according to the anatomical seat and clinical symptoms of the disease, although probably an instance of true myelogenic leukæmia has not yet been observed.*

* Several years ago, the author had under observation in Professor Nothnagel's clinic a case of nephritis in which the blood contained leucocytes of great size and with unusually large nuclei, their proportion to the red corpuscles (as determined by a single examination) being as 1 : 50. The autopsy revealed, in addition to chronic nephritis, changes in the medulla of the bones, like those described by *Neumann* in connection with leukæmia.

When large and small leucocytes—the latter preponderating—are found in the blood, the leukæmia is of a lymphatico-splenic character. When, on the other hand, the larger cells alone are found, we are justified in concluding that the disease is splenic, with but little involvement of the lymphatic glands or marrow. If many corpuscles of a transitional form are found, nucleated red cells, and especially large white multinuclear corpuscles, there remains no doubt that the bone-marrow is the seat of serious changes, and the disease is of the myelogenic type.[39]

Crystals are occasionally found in leukæmic blood (*Charcot*, *Robin*, *Vulpian*).[40] *Neumann*[41] attributes their origin to the marrow, and describes them as colourless, shining, oblong octahedrals (*Ph. Schreiner*).[42] They are seldom met with. The author has often sought, but never

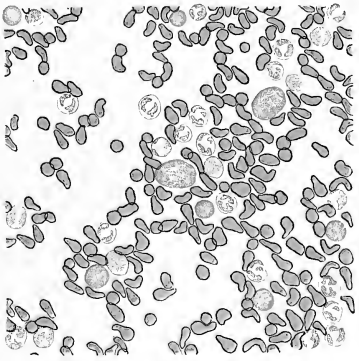


Fig. 12.—Eosinophile Cells from Leukæmic Blood.

seen, them in freshly-drawn blood. It may be that they form only in blood that has been allowed to stand.[43] They are doubtless identical with those sometimes discovered in the sputum and fæces, and constantly in the seminal fluid.*

It should also be mentioned that the red blood-corpuscles commonly undergo changes of form in leukæmia, a condition which first received the name of **Poikilocytosis** from *Quincke* (p. 24).

An advanced stage of leukæmia is readily recognised by the aid of the microscope, but it is by no means easy to distinguish between commencing leukæmia and a pronounced leucocytosis; and this is a task which the physician will often have to undertake. *Magnus Huss*

* For the chemical constitution of these crystals, see the chapters on the discharges referred to.

and others[44] employ the term "leukæmia" only when the white corpuscles are in a proportion not less than 1 : 20.

We are indebted to *P. Ehrlich*[45] for an excellent means by which we may recognise an early stage of leukæmia. By observing the "granules" present in the protoplasm of the white corpuscles, he found that these uniformly exhibited remarkable differences in their staining properties—differences which have both a physiological and a pathological significance. Upon this basis he distinguishes five several varieties of what he called "granules," classing them as α to ε granules. In all cases of acute leucocytosis, the mono- and poly-nuclear forms which furnish the ε granules are alone increased in number, whilst the α granules—which are also styled "eosinophile," from their property of taking up eosin—are apparently fewer. Precisely the reverse of this, again, obtains at the beginning of leukæmia, and the fact provides us with a good means of diagnosing that condition in its early stage. The method of examination is as follows:—The blood is spread in a very thin layer between two cover-glasses, which are then grasped with forceps, dried in an exsiccator, and heated upon plates of copper foil (for this purpose a drying chamber for temperatures beyond 100° C. will serve very well *) for a considerable time (10 to 12 hours) at 120°–130° C. A drop of concentrated eosin-glycerine solution is then added to the preparation, the colouring matter is washed off with water, and the preparation dried, and examined in Canada balsam or oil of cloves.

According to *Huber*,[46] good results may also be obtained in the following manner:—Two grms. each of aurantia, indulin, and eosin are dissolved in 30 grms. of glycerine, the resulting thick fluid well shaken up, and the cover-glasses (dried and heated as above for several hours at 120° C.) are immersed in it for a period varying from half an hour to some days. When taken out, they are carefully washed with distilled water, dried in the air, and examined in Canada balsam or dammar varnish. Should the case be one of early leukæmia, the red blood-corpuscles in such a preparation are stained a reddish-yellow, the nuclei of the white corpuscles have taken up the blue colouring matter, and in addition there are seen large leucocytes (eosinophile cells) distended with granules of a deep red tint (eosinophile granules). In such preparations the latter are also commonly to be seen scattered separately or associated irregularly in groups (fig. 12), together with large colourless cellular bodies of oval shape, distended at their poles with similar particles. (See Appendix VI.)

During the last few years the author has repeatedly examined the

* Hardening with absolute alcohol may also be employed; then, of course, it will be necessary to drive off the alcohol before staining.

blood of healthy and anæmic, and especially of rickety children, for the appearances described here. It seems that these structures occur singly and not in large numbers in the normal blood of such individuals, and the same is the case in anæmic conditions of all sorts. In one instance alone were they found in great abundance, and that was the case of a lad who suffered from tuberculosis, but not from leukæmia. If it follows from these researches that the detection of eosinophile granules in increased numbers in the blood cannot be taken absolutely as a sign of commencing leukæmia, their presence will nevertheless serve, in most cases, to warn the physician that he has to deal with an early stage of that disease.

4. Melanæmia.[47]—The microscopical appearance of the blood in this very rare disease suffices for its recognition. There are to be seen floating amongst the blood-corpuscles smaller or larger granules and granulation masses, possibly pigment lumps, which are usually black, more seldom brown and yellow. The granules are united to each other by a substance which is soluble in acids and alkalies.

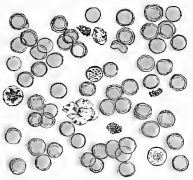


Fig. 13.—Melanæmic Blood.

The pigment occurs also in the form of separate granules equal in size to leucocytes. Finally (and this, in the author's opinion, is the commonest case), the pigment particles, both small and large, may be enclosed within cells which occasionally resemble white blood-corpuscles, and sometimes differ from them in being flask- or spindle-shaped. The pigment lumps are very rarely observed; but, on the other hand, the granules, and, more commonly still, pigment-laden white corpuscles, are often temporarily present after a severe attack of ague and in relapsing fever. The preparation (shown in fig. 13) was made from the blood of a man who had suffered for a year from malarial disease which he had contracted in the tropics.

5. Microcythæmia.—This condition was first described by *Vanlair* and *Masius*.[48] It is characterised by the presence in the blood of small hæmoglobin-containing elements (*microcytes*), which are probably derived from the red-blood cells, and are generally smaller, but occasionally larger (*megaloblasts* of *Hayem* and *Ehrlich*) than these.

Such bodies are found in many morbid states, and notably in toxic states, infectious diseases, pronounced anæmia, and in burns. Notwithstanding that much has been written about them, there is really but little known on the subject of microcytes, and their discovery is of no assistance in diagnosis. *Litten* has shown that they may rapidly appear in the blood, and as rapidly disappear from it. We may refer here also to *Bettelheim's* [49] observations on the subject of minute mobile granules in the blood. *Gram* and *Gräber* [50] regard the microcytes as the result of postmortem changes in the blood. The latter believes that they represent the final product of the rapid and uniform abstraction of water from the corpuscles; and since such a process will take place most readily in blood which contains but little water (and is, therefore, relatively rich in albumin), the subject of microcythæmia is invested, by this theory, with a certain practical importance.

6. Poikilocytosis.—By this term it is usual to describe an affection of the blood in which the red corpuscles exhibit a remarkable

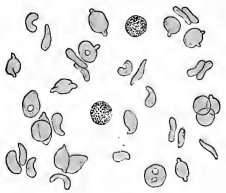


Fig. 14.—Poikilocytosis of the Blood.

variety as to form and size.[51] The condition was first noticed in pernicious anæmia, and has been regarded by some authors as pathognomonic of that disease. Cases, however, have been recorded (*Grainger Stewart, Lépine, Hermann Müller*) in which no such changes were found.

The appearance of the red blood-corpuscles in this condition exhibits a remarkable variety. Some are normal in shape and size, but amongst them are seen smaller forms and others of great size (megaloblasts). Some, again, are flask-shaped, and often tipped at one extremity with a little knob; while others of irregular outline have been compared to such different objects as an anvil, a biscuit, a goblet, or a kidney (fig. 14). *Friedreich* and *Mosler* have seen amœba-like processes on the red corpuscles. The author has also observed similar appearances, and inclines to the belief that the prominent features of poikilocytosis depend upon the fact that the red corpuscles are endowed with an abnormal power of contractility. From the above description it may be seen that poikilocytosis is easily distinguished with the microscope.

Such appearances are not confined to any one disease, but are common to many in which the blood is greatly altered, and especially in respect of the diminution of its cellular elements and of the red corpuscles in particular.

The author has himself observed them in cases of chlorosis and severe anæmia of whatever kind, and typically developed in pernicious anæmia. He has also seen them in the cancerous cachexia, in leukæmia, and in amyloid degeneration of the viscera. *Gräber* believes that the poikilocytes do not exist in the circulating blood; a view which the author does not assent to in all cases.

7. Changes in the Formed Elements of the Blood in Chlorosis.[52]—Although the minute examination of the blood in chlorosis commonly discloses few or none of the changes previously described, it will prove both interesting and profitable to mark those particulars in which it differs from simple oligocythæmia on the one hand, and from pernicious anæmia on the other.

Chlorotic blood is especially characterised by its paler colour, not necessarily accompanied by other change in the physical properties of its constituents. Microscopical examination shows usually that the red corpuscles are abnormally pale without their numbers being markedly diminished. The application of the methods for counting the corpuscles and for estimating the amount of contained hæmoglobin proves, in the greater proportion of cases, that while the number of corpuscles is but slightly altered, the blood is very deficient in hæmoglobin. Generalising somewhat, the conditions that obtain in chlorosis may be said to be:—Diminution of the amount of hæmoglobin, together with a less diminution of the cellular elements of the blood, and with or without an accompanying relative increase of leucocytes. Poikilocytosis is a frequent phenomenon in chlorotic blood, whilst microcytes and megaloblasts are also not of rare occurrence.

8. Changes in the Formed Elements of the Blood in Pernicious Anæmia.[53]—Very different is the condition witnessed in pernicious anæmia. The blood is thin, light-coloured, and shows all the changes which have already been seen to belong to oligocythæmia. Under the microscope its cellular elements are seen to be enormously diminished in quantity, a condition which is seldom or never observed in the very worst forms of simple anæmia. The number of blood-cells, according to *Laache*, may fall so low as 360,000 to the cubic millimetre. Further, the individual red cells are often larger than normal, and exhibit in an exquisite manner the characteristics of poikilocytosis.

To estimate the diameter of the corpuscles, the method of "dry measurement" may be employed. It is founded upon the fact observed by *C. Schmidt*, that red blood-corpuscles, when rapidly dried, retain

their form permanently. According to Laache's directions, the proceeding is as follows :—A slide is gently heated and then swept quickly and lightly over the surface of a very small drop of blood drawn from the skin. The blood dries rapidly, and when placed under the microscope, the corpuscles appear as bi-concave discs, lying apart from one another, so that their diameter can easily be measured with the familiar micrometric apparatus (micrometer eye-piece). In the case of healthy blood corpuscles, the diameter ranges between 6.5 or 6.7 and 9.0, 9.4 μ.* In the blood of pernicious anæmia, red corpuscles are found of 10–15 μ diameter. Microcytes are seldom found in the blood in this disease.

An important sign of pernicious anæmia, as *Hayem* first showed, is the fact that the number of red cells in the blood is inversely proportional to the quantity of hæmoglobin which they contain. *Copeman*[54] made the remarkable discovery that when the blood of pernicious anæmia is quickly dried, rhombic crystals of hæmoglobin are sometimes formed.

The presence of amorphous hæmatoidin in fresh blood is not a very rare occurrence. The author met with it in the case of a child of four months suffering from hereditary syphilis and severe icterus.

We see then that the characteristic features of the blood in pernicious anæmia are : a general **diminution** of its cellular constituents, with a relative **increase** in the size of the red corpuscles and in the quantity of hæmoglobin which they contain.[55] All these properties can be shown by the methods described above.

V. THE PARASITES OF THE BLOOD.—Amongst these are members both of the vegetable and of the animal kingdom.

A. Vegetable Parasites.—We shall follow the plan usually adopted in clinical medicine, by which micro-organisms are divided into three great groups—(1.) Moulds; (2.) Yeasts (Saccharomycetes); and (3.) Fission-fungi (Schizomycetes, bacteria). The third group alone concerns us here, for to it belong, almost without exception, the parasites which as yet have been found to infest the blood. Yeast cells, indeed, have occasionally been seen in the blood of animals,[56] but not yet in that of man, nor has any definite disease been attributed to their agency. We have, therefore, to describe the bacilli of *anthrax*, of *relapsing fever*, of *tubercle*, *glanders*, and of *typhoid*. With reference to the bacillus of *tetanus*, the recent investigations as to its occurrence and significance are to be received with great reserve. The statements concerning the

* The Greek letter μ represents one-thousandth of a millimetre (μ=0.001 millimetre), and is the sign of a micromillimetre or micron.

presence of streptococci in the blood of certain diseases also need confirmation before they are made the basis of clinical inferences.

Methods of Examining the Blood for Micro-organisms.—In certain diseases (as, *e.g.*, of relapsing fever and anthrax) no difficulty will be found in determining the presence of bacilli in the blood by means of a simple microscopical examination. In other instances (and especially in miliary tuberculosis, typhoid, and glanders) we must resort to the methods of *Koch* and *Ehrlich*. In all alike the common process is to dry the blood in a thin layer, so that the shape of the corpuscles is lost without altering the appearance of the bacilli, and then to submit it to the staining methods employed by *Koch*, *Ehrlich*, *Weigert*, &c.,[57] and other investigators. *The general principle of the methods is this, that the fungi are deeply stained by the basic aniline dyes.* To these basic aniline dyes belong:—Bismarck-brown, vesuvin, aniline-brown, fuchsin, methylene blue, gentian-violet, and methyl-violet. It must be observed, however, that other substances besides the micro-organisms are coloured by these dyes. Thus, for instance, masses of protoplasm, cell-nuclei, and the products of cellular disintegration stain readily with them. This is true also of the γ and δ granules of *Ehrlich*, and, in fact, they have over and over again been mistaken for fungi.

Preparation of Cover-glasses.—The skin of the finger-tip from which the blood is to be taken must first be thoroughly cleansed with soap and water and a nail-brush, and washed with corrosive sublimate (1 in 1000). All traces of the latter are removed by first rinsing the finger with alcohol, and finally pouring æther upon it. A pretty deep puncture is now made in it with a needle previously sterilised by heat. The first drops of blood that issue are swept away with a well-sterilised platinum needle, and a cover-glass held in steel forceps also sterilised is quickly brought in contact with the surface of the blood as it flows freshly from the wound. To this cover-glass another is at once applied, and the blood spread in a very thin layer between them. They are then separated with the aid of two pairs of forceps, and placed to dry in an exsiccator or in a chamber free from draughts and dust. Care should be taken that the cover-glasses employed are absolutely free from impurities, and to this end they should have been washed before use in corrosive sublimate, alcohol, and æther. Not less care must be taken to secure the cleanliness of the needles and forceps used. When dried in this manner, the cover-glass, with the prepared surface upwards, is three times passed cautiously through the flame of a Bunsen's burner, and then kept for some hours at a temperature of 120°. After this a drop of a strong watery solution of a basic aniline dye is applied to it by means of a pipette, allowed to rest on it for a short time—some minutes at the longest—and then washed off with a gentle stream of sterilised

distilled water, directed so that it flows over, but not directly down upon, the stained parts. With this object the cover-glass should be held obliquely to the stream. The preparation can be examined whilst wet. To mount it in Canada balsam, dammar varnish, or oil of cloves, it is necessary to dry it again before placing it with a drop of one of these substances on a slide.

If too concentrated a staining fluid has been used, and the specimen proves too highly stained, the excess can be removed by the addition of alcohol.* *Ehrlich* [58] claims for methylene blue the advantage that, even when submitted to its action for a long time, the preparation will not be too deeply coloured.

Over-staining is less likely to occur when alcohol, glycerine, or acetic acid has been added to the water in which the dye is dissolved. It must be mentioned, however, that vesuvin, Bismarck-brown, and aniline-brown cannot be employed in alcoholic solution.

Further, it is a good plan, especially for the purposes of a provisional inspection, to treat the cover-glass directly by placing upon it a drop of a filtered alcoholic solution of an aniline dye, such as fuchsin or methyl-violet, and then to wash off the superfluous staining fluid with alcohol, when the preparation may be examined in the manner already described.

With reference to the staining fluid, it is advisable to prepare it afresh each time it is required, because it readily decomposes when allowed to stand; and, besides, fungus vegetation is apt to thrive rapidly in dilute solutions. To examine a dry cover-glass preparation of blood for micro-organisms, the directions of *Löffler* [59] may be followed with advantage:—The cover-glasses, prepared as above, are left for 5–10 minutes in a staining fluid consisting of 30 cc. of a concentrated alcoholic solution of methylene blue, and 100 cc. of 1 : 10,000 solution of potash, then washed for 5–10 seconds in $\frac{1}{2}$ per cent. solution of acetic acid, treated with alcohol, dried, and mounted in oil of cloves or Canada balsam.

Gram's [60] method, which is also to be commended for the examination of micro-organisms, is somewhat different. He prepares the cover-glass in the manner described above, and places it for some minutes in an *Ehrlich-Weigert* solution of gentian-violet and aniline water (p. 76). When sufficiently stained, he puts it into a solution of iodine and iodide of potassium (iodine 1.0, iodide of potass. 2.0, distilled water 300.0), when a dull red-brown precipitate forms. It remains in the iodine-potassic-iodide solution for two or three minutes. It is then put to bleach in absolute alcohol, where it remains for some time. When taken out and examined, all the cellular elements are seen to be colourless,

* Glycerine or dilute acetic acid will serve this purpose also.

with the exception of the micro-organisms, which are deeply stained a dark blue.

Günther's method of investigation for the spirillum of relapsing fever (see p. 31) is also applicable to the examination of blood for micro-organisms. The microscope employed in these researches should be provided with an oil-immersion lens, and an Abbe's reflector and open condenser (Chap. X.).

1. Bacillus of Anthrax.—The presence of micro-organisms in the blood of men and animals suffering from anthrax was pointed out by *Pollender*, *Brauell*, and *Davaine*.[61] Many other observers (*Buhl*, *Waldeyer*, *E. Wagner*, and *W. Müller*[62]) have since described the occurrence of the bacillus of this disease in human blood; but in all cases the number as seen in man falls far short of that witnessed in the case of animals, and it varies according to the part from which the blood is taken. It is found most abundantly in splenic blood. When

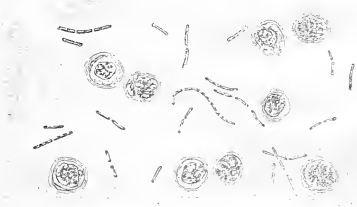


Fig. 15.—Anthrax Bacilli from a Rabbit's Blood.

looked at through the microscope, the micro-organisms are seen to be motionless, rod-like bodies, 5–12 μ long,* and almost uniformly 1 μ broad, slightly thickened at the extremities, and occasionally having the appearance of transverse segmentation towards the middle. Even in unstained preparations they can be readily seen when they are present in great numbers.

The blood is at the same time thin and of a dark red colour; commonly, too, pronounced leucocytosis co-exists. Where typical anthrax bacilli are found in the blood, the diagnosis of the disease is established beyond question; but, on the other hand, it must be borne in mind that the symptoms of anthrax may be well marked while no bacilli are anywhere to be seen. In such a case the diagnosis may be established by injecting some of the suspected blood into an animal (mouse or guinea-pig), which, if anthrax be present, will shortly exhibit symptoms of the disease, and its blood when examined will be found

* μ=0.001 mm.

to hold bacilli in profusion (fig. 15). The bacillus of anthrax does not form into long threads either in the blood or in the living tissues, and it has no spores (*R. Koch*).[63] It increases only by division. Fig. 15 represents bacilli from a rabbit's blood.

Fig. 16 is from a preparation made from the blood of a man who had died of anthrax (wool-sorter's disease). A comparison of figs. 15 and 16 will serve to make the bacilli evident.

The examination of the blood in anthrax is conducted in the manner described above (for dry cover-glass preparations and staining with basic aniline dyes). Löffler's method is especially applicable to the purpose. It should be mentioned here that the affection known as "wool-sorter's disease," the nature of which was formerly so obscure, is now known (from the observations made by *Eppinger* and *R. Paltauf*[64])

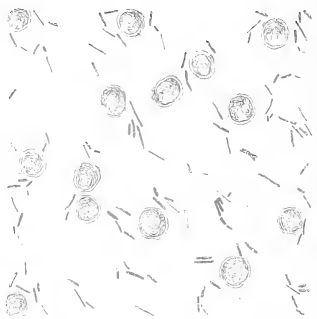


Fig. 16.—Anthrax Bacilli from Human Blood.

to be identical with anthrax, and when the clinical symptoms of that disease come under observation, the physician should in all cases examine the blood.

2. Spirillum of Relapsing Fever.—The spirillum was first noticed by *Obermeyer*[65] in the blood of a patient suffering from relapsing fever. It has since been seen by many observers, but most authorities are agreed that it is to be found only during the paroxysms of the disease, and that as the temperature falls the bacilli disappear.* When a specimen of blood containing them is placed under the microscope, the bacilli appear as long and very delicate unsegmented threads twisted into spirals. Their average length is about six or seven times the diameter of a red blood-corpuscle. They have a

* In opposition to this view, see *Naunyn*, Centralbl. für Bacter. und Parasitenk., iv. 376, 1888.

brisk vibratile movement in the direction of their long axis. This motion, when the blood is examined with a low power, gives to the eye a peculiar impression of disturbance, and will immediately lead the practised observer to look for the presence of spirilla. If he then increases the power, and, still better, if he employs an oil immersion-lens with Abbe's condenser and a small diaphragm, the spirilla come clearly into view. These bodies are extremely sensitive to reagents of all kinds. Even the addition of distilled water will cause them to disappear.

The number of spirilla which are to be seen together in a specimen of blood varies greatly, and often bears no relation to the intensity of the fever.

If the blood is examined in the intervals of the disease, provided another paroxysm be impending, it displays peculiar refractive bodies resembling diplococci, which are especially numerous when the paroxysm

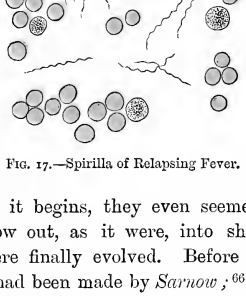


Fig. 17.—Spirilla of Relapsing Fever.

sets in; and just as it begins, they even seemed to the author in certain cases to grow out, as it were, into short thick rods, from which the spirilla were finally evolved. Before those of the author, similar observations had been made by *Sarnow*;[66] and pending further confirmation, it seems probable that these bodies are the spores of spirilla, which have so long been sought for.

Since both the spirilla and the forms just mentioned have been met with as yet only in the blood of persons suffering from relapsing fever,* their great importance as a clinical test is sufficiently apparent.

For the purposes of research an unaltered specimen of blood serves best, but dried preparations can also be made and stained most appropriately with fuchsin.

Günther[67] has recently advocated the following plan:—The cover-glasses, prepared in the usual manner, are immersed for ten seconds

* Bodies resembling them are found in the buccal secretion (*vide* chapter on the buccal fluids).

in a 5 per cent. solution of acetic acid, to destroy the colour of the red corpuscles. The acetic acid is then removed as far as possible by blowing upon the cover-glass, and its last traces neutralised by holding the preparation surface over an open flask of strong ammonia solution (shaken up previously). The preparation is next stained in the *Ehrlich-Weigert* aniline-water gentian-violet solution, and the staining fluid washed off. It is then mounted in Canada- or xylol-balsam before it is examined. This method is a very good one for the examination of the blood for micro-organisms in general.

3. Tubercle Bacillus.—This was first described by *Weichselbaum*[68] as occurring postmortem in a case of miliary tuberculosis. To one of his pupils named *Meisels*[69] belongs the credit of its first discovery in the same connection during life. Since then much knowledge on the subject has been derived from the labours of various observers (*Lustig*, *Sticker*, *Doutrelepont*, *Rütimeyer*).[70]

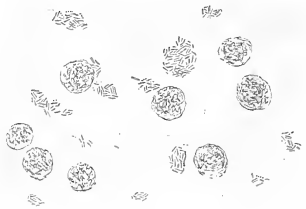


Fig. 18.—Tubercle Bacilli in the Blood.

The number of tubercle bacilli found together is commonly small. Indeed, when present, they are often so few as to baffle the most careful investigation. It rarely happens that a specimen of blood is found so rich in bacilli as that represented in fig. 18; but, when detected, they leave no doubt of the existence of general miliary tuberculosis.

The mode of research is the same as that detailed at p. 75 for the examination of the tubercle-bacilli of the sputum, and the cover-glass is prepared in the manner already described.

4. Bacillus of Glanders.—The bacillus of glanders was first discovered by *Löffler*[71] and *Schütz*,[72] and its association with the disease has recently been confirmed by *Israel*[73] and *Weichselbaum*.[74] The bacilli are rod-like bodies, 2–3 μ long and 0.3–0.4 μ broad, often carrying a spore at the extremity. They are found in the farcy buds and ulceration of glanders, and also in the blood of persons suffering from that disease. Fig. 19 represents the bacilli seen in a specimen of blood taken from a case of glanders in the Vienna general hospital.

They may be shown in the blood by means of the dried preparation, and they are best stained by Löffler's method for detecting these fungi. (See Chapter VIII.)

5. Bacillus of Typhoid Fever.—Bacilli have of late years repeatedly been found in the blood of typhoid patients, and they are doubtless the exciting cause of the disease.[75] *Rütimeyer* and *Neuhauss*[76] experimented upon the blood taken from the roseolar spots of typhoid patients, and succeeded in cultivating bacilli from it. Further information on this subject will be found in the chapter on *Fæces*.

6. Streptococci.—*V. Noorden*[77] recently found streptococci in blood taken from the body of a woman who had died of erysipelas. They exhibited in their cultivation-properties the closest resemblance to the well-known streptococci of *Fehleisen* and *Rosenbach*. Further researches in connection with the disease from which they were obtained will serve to determine their value as a factor in diagnosis.

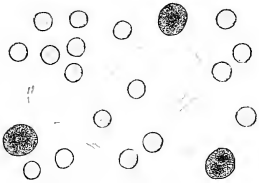


Fig. 19.—Bacilli of Glanders in Human Blood.

[*Streptococcus Pyogenes.*—A chain-forming micrococcus was described by *Ogston* as occurring in abscesses. It was afterwards submitted to Koch's cultivation processes by *Rosenbach*, who gave it the name of Streptococcus pyogenes, and it is now known to be associated with septic processes of all kinds, whether in men or animals. It is apparently the same as *Fehleisen's* S. erysipelatis and *Löffler's* Diphtheriacoccus. It has been obtained from the pus of pyæmic abscesses and empyema, from the tissue-fluids in spreading gangrene, from the vesicles and pustules of small-pox, and in contaminated calf's lymph. It occurs in the blood-vessels in certain cases of diphtheria, scarlet fever, puerperal septicæmia, measles, and typhoid; but it must be regarded only as a secondary result of those affections. It may gain admission to the system in any disease attended with lesions to the skin or mucous membrane, setting up destructive processes, when the resistance of the tissues is greatly impaired by the working of a special virus (*Crookshank*).[78]

Streptococcus of Scarlatina.—*Dr. Klein* has recently succeeded in separating a micro-organism obtained from the blood of scarlatina patients, which he regards as the contagium, virus, or actual cause of that disease. When cultivated by inoculating the surface of nutrient gelatine with the postmortem blood, the micro-organisms bear a close resemblance to the Streptococcus pyogenes. Klein is of opinion that the two are sufficiently distinguished, both morphologically and by their pathogenic effects upon animals. The experiments upon which he relies, however, are questioned by *Crookshank*,[79] who maintains the identity of the

micro-organisms concerned. A Streptococcus which Klein believes to be the same as that of scarlatina was found in the blood of cows in an outbreak of disease amongst those animals at Hendon; and this, when inoculated upon calves, produced in them the postmortem appearances of scarlatina. Moreover, the cow-disease alluded to is thought to have caused an epidemic of scarlet fever amongst men through the medium of the milk.[80]]

7. Micro-organism of the Blood in Hydrophobia.—*Bareggi*[81] found constantly present in the blood in hydrophobia a micro-organism which stained with methylene blue. Placed on slices of potato at 25°–27° C., it developed in the course of forty-eight hours in flattened hemispherical cultivations, of a colour ranging between whitish-grey, yellow, and citron. In test-tube cultivations (see the chapter on *Bacteriological Methods*), it behaved like the bacillus of Asiatic cholera. Further research is needed to establish a connection between this micro-organism and hydrophobia.

8. Bacillus of Tetanus.—Many authorities[82] are of opinion that tetanus is an infectious disease, produced by the agency of the "bristle-bacillus" of Nikolaier. According to the observations of the latter investigator, the bacilli in question are somewhat longer and thicker than those of mouse-septicæmia. They sometimes form in threads, commonly in irregular heaps, and occasionally produce spores. The bacilli themselves, or their spores, have been found in the blood (?) (*Hochsinger*). They stain readily in dry cover-glass preparations, and may be cultivated outside the system. The fact may be mentioned here that from cultivations of the bacillus *Brieger*[83] has isolated several ptomaines—tetanin, tetanotoxin, and spasmotoxin—with which he has induced tetanic poisoning symptoms in animals; and more recently the same observer has obtained similar poisons from the blood of persons who had died of tetanus.

In spite of these discoveries, however, we are not yet in a position to say that the bacillus is the cause of tetanus. It is only necessary to cite the well-established authority of *Flügge*, in whose laboratory experiments on the subject have recently been conducted, and he sums up their result in the statement that the bacillus cannot be held to be either uniformly or exclusively present in tetanus. The researches of *Belfanti* and *Pescarolo*[84] point to the same conclusion.

9. Bacillus and Plasmodium Malariæ.—*Klebs*[85] and *Tommasi-Crudeli* regard a distinctive bacillus which they have found in the soil of the Campagna as the specific cause of malaria. *Marchiafava* and *Celli*[86] found amœboid bodies (plasmodia), often containing granules and masses of black pigment, enclosed within the red blood-corpuscles of persons suffering from malarial poisoning. These bodies stain with methylene blue. They have not yet been cultivated outside the human system, but by inoculation and the intravenous injection of malarial blood containing them, Marchiafava and Celli have succeeded

in communicating the disease to other individuals, and the infected blood in its turn exhibited the plasmodia.[87] The statements of these authors are borne out by others.[88] *Metschnikoff* proposes to name the parasite Hæmatophyllum malariæ. *W. Osler* has examined the blood in seventy cases of malarial disease, and found it present in all. It would appear from his researches, however, that the organisms concerned assume a greater variety of forms than was previously supposed.

Councilman,[89] again, has recently supported the views of Lavaran, Marchiafava, and Celli. He describes several forms of the parasite, and opposes the doctrine of *Mosso*,[90] who sought to prove by his experiments that the plasmodium resolves itself into degeneration types of the red blood-corpuscles; and maintains that similar appearances can be found in the blood apart from malaria. Finally, *L. Pfeiffer*[91] has also seen such in the blood after inoculation with vaccine and in scarlatina. *Tommasi-Crudeli*,[92] on the other hand, regards the forms in question rather as the result than as the cause of malarial disease, believing them to be the product of the degeneration of the red blood-corpuscles, and further maintains that the bacillus discovered by Klebs and himself is the true cause of the disease. This opinion is shared by *Schiavuzzi*,[93] who still more recently has succeeded in cultivating the micro-organism outside the system. The theory of *Danilewsky*[94] remains to be mentioned. That author supposes that the bodies under discussion are identical with the hæmatozoa observed in the blood of many birds.

It will be seen from so many contradictory statements that the question as to what is the specific cause of intermittent fever is not yet satisfactorily solved. Too much importance must not be attached as an element in diagnosis to any of the appearances described. The author has many times examined the blood of patients suffering from intermittent fever, and he has never seen such bodies as those described by Marchiafava, Celli, and others. It may be that they occur only in the severe forms of the disease, which rarely come under observation.

It remains to be noticed that *Loeff* and *Pfeiffer*[95] have discovered in the blood in SMALL-POX certain protozoa, to which they attribute a pathological significance.

B. Animal Parasites (*Hæmatozoa*).—Under this heading we have to describe Distoma hæmatobium and Filaria sanguinis hominis. Both are worms—the first of the class *Platodes* and order *Trematodes*;[96] the second of the class *Annelides*, order *Nematodes*, family Filariadeæ.

1. Distoma Hæmatobium (fig. 20).—*Bilharz*[97] has found this parasite in the main trunk and branches of the portal vein, in the splenic and mesenteric veins, and in the venous plexuses of the bladder and rectum. As the result of his investigations, he came to the conclusion

that more than half the adult Fellaheen and Coptic population of Egypt suffered from it. Except in the blood, the eggs are more commonly found than the parasite itself, and this in the liver, the lungs, the bladder, the urethra, the large intestine, and the urine (see chapter on *Urine*), giving rise to diarrhœa, hæmaturia, and ulceration of the mucous surfaces of these organs. Distoma hæmatobium has not yet, so far as we know, been found in the peripheral blood-vessels, and on this account it is seldom seen in the microscopic examination of the blood during life. The parasite is of a white colour. The male and female are distinct individuals. The former is from 12–14 mm. long, the latter 16–19 mm. The male is thicker than the female. There are oral and abdominal suckers anteriorly, and the genital opening in either sex lies close behind the latter. On the abdominal aspect of the male is a deep trench with overlapping edges, which begins just behind the abdominal pore, and serves for the reception of the female.

FIG. 20.—Distoma hæmatobium.

Male and Female; Eggs.

The eggs are slender bodies, about 0.12 mm. long and 0.04 mm. broad, and furnished with a little spike, which projects from the extremity or at the side.

Ringer[98] discovered a new form of this worm at Tamsui in Formosa, and *Manson*[99] found the eggs of the same species in the bloody sputa of a Chinese who had lived for a long period in Formosa.

2. Filaria Sanguinis Hominis.—*Wucherer*[100] is said to have first discovered this parasite in Bahia. *Dr. Lewis*[101] of Calcutta, at all events, was the first to describe it as occurring in the living body.

It is the larva of a filiform worm, which in the mature state measures about 40 mm. in length. According to *Bourne*,[102] the male is $1\frac{1}{4}$ in. long, and carries two spiculæ. The larva, as it is found in the blood of the living subject, is 0.0075 mm. in breadth, and 0.34 mm. long. It has a short rounded head, with a tongue-like appendage, and a long pointed tail. From the hinder extremity a ribbon-like process projects, and when viewed with a high power of the microscope, this, like the cephalic appendage, is seen to be the end of a closed sac in which the animal can coil or extend itself. This envelope is entirely structureless, but the contained parasite is seen under a very high power to be

transversely striated and very granular. In the blood the animal exhibits for hours at a time the liveliest movements.[103] At first it seems to be homogeneous and transparent, but after some hours its motion ceases, it assumes a darker tint, and the granular contents of its body are easily discernible.

The parasite is rarely found elsewhere than in the blood and lymph of persons who live, or have lived, in the tropics. It has recently, however, been met with in temperate climates.[104]

It may infest the blood for months, or even years, without giving any sign of its presence; but commonly, by blocking or perforation of capillaries and lymphatics, it leads to hæmaturia and chyluria, or to hæmorrhage and lymphatic exudation in different organs.

It would seem that the parasite is often conveyed by mosquito bites.[105] *Patrick Manson*, *Stephen Mackenzie*, and others[106] have shown that in persons who suffer from the presence of Filaria sanguinis hominis, the

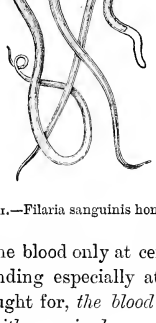


Fig. 21.—Filaria sanguinis hominis.

parasite is to be found in the blood only at certain periods, being absent during the day, and abounding especially at night. Hence it is important that, when it is sought for, *the blood should be taken from the patient at night, and forthwith examined.*

VI.—CHEMICAL CHANGES IN THE BLOOD.

1. Colouring Matter.[107]—The most important constituent of the blood is the **oxyhæmoglobin**—the combination of the colouring matter with oxygen which is formed by the aëration of the blood in the lungs. The characteristic spectrum of dilute solutions of this body exhibits two absorption-bands between D and E (of *Fraunhofer's* lines). The band nearest to D is darker, narrower, and more strongly marked; that next to E is less sharply defined and broader (fig. 22).

When submitted to the action of deoxidising substances, oxyhæmo-

globin gives place to **Reduced Hæmoglobin.** The spectrum of this body is characterised by a single band, occupying a space about midway between the former two bands (fig. 23).

Under the action of acids of all kinds, of strong alkalies, and even

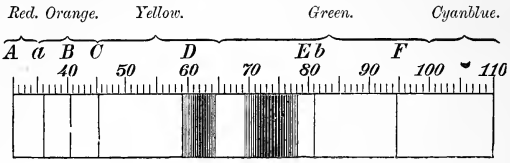


Fig. 22.—Spectrum of Oxyhæmoglobin.

of CO_2, hæmoglobin is broken up into (1) a proteid resembling *globulin*, and (2) the iron-containing body known as **Hæmatin.**

The spectrum of hæmatin in *alkaline* solution shows an absorption-band lying between C and D of Fraunhofer's lines (fig. 24). In an

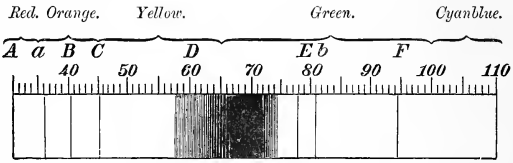


Fig. 23.—Spectrum of Reduced Hæmoglobin.

acid solution its spectrum is identical with that of methæmoglobin in acid solution (fig. 27).

Hæmatin in alkaline solution, when treated with reducing agents, yields **Reduced Hæmatin.** The spectrum of this body (fig. 25) exhibits

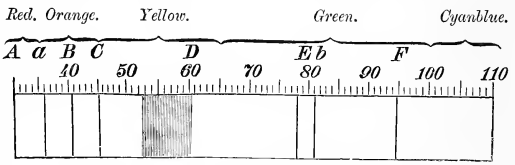


Fig. 24.—Spectrum of Hæmatin in an Alkaline Solution.

two absorption-bands between D and E. If the reduced solution be shaken up with air or oxygen, these bands disappear again, and the spectrum shows once more the two absorption-bands of the alkaline solution of hæmatin.

Hæmatin in combination with hydrochloric acid forms, even from

minute blood-traces, microscopic highly characteristic, brown, rhombic crystals (fig. 26). These crystals were first discovered by *Teichmann*.[108] They are of the utmost importance, inasmuch as their formation affords an admirable test for blood-colouring matter under the most varied conditions. We shall repeatedly have to revert to this later on.

To test for hæmin crystals, the following plan may be adopted:—The substance supposed to contain blood-colouring matter must be

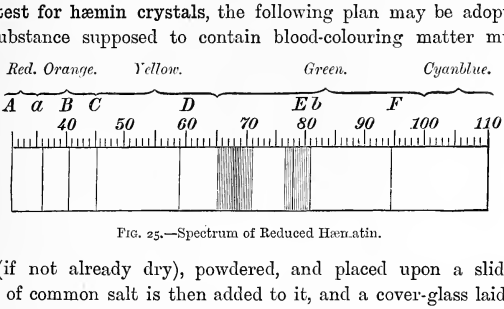


FIG. 25.—Spectrum of Reduced Hæmatin.

dried (if not already dry), powdered, and placed upon a slide. A crystal of common salt is then added to it, and a cover-glass laid upon the preparation. A few drops of glacial acetic acid are then allowed to flow beneath the cover-glass. The whole is heated to a point below boiling, and after a little while, if the substance contains blood-colouring matter, hæmin crystals (fig. 26) will be discernible by the microscope.

When an acid solution of hæmatin in alcohol is treated with reducing agents, a series of colouring-matter derivatives are obtained. Of these, *hæmatoporphyrin*

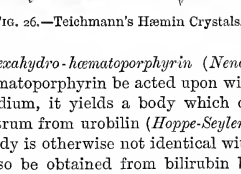


FIG. 26.—Teichmann's Hæmin Crystals.

(*Hoppe-Seyler*[109]) and *hexahydro-hæmatoporphyrin* (*Nencki-Sieber*[110]) have been already isolated. If hæmatoporphyrin be acted upon with tin and hydrochloric acid in an alcoholic medium, it yields a body which cannot be distinguished chemically or by its spectrum from urobilin (*Hoppe-Seyler*).[111] According to *C. le Nobel*,[112] however, this body is otherwise not identical with urobilin.

This substance can also be obtained from bilirubin by the action of sodium amalgam (*Maly*).[113] There is another important derivative of hæmatin, which is apparently identical with bilirubin. This is **Hæmatoidin**, a substance first discovered by *Virchow*[114] in extravasated blood. It was afterwards found in old cerebral clots, in splenic infarctions, blood cysts, &c., and it has been met with in human urine, in the sputum, and fæces.*

* See the chapters on these subjects.

From these facts, namely, that urobilin can be formed from hæmatin by the action of reducing agents, and that this substance can also be formed from bilirubin, *Neneki* and *Sieber* have established very simple relations between the colouring matter of the blood and that of the bile. They have constructed a new formula to express the constitution of hæmatin, and from this it would appear that hæmatin is changed into bilirubin by the addition of two molecules of water and the removal of one atom of iron, thus:—

$$\underbrace{C_{32}\ H_{32}\ N_4\ O_4\ Fe}_{\text{Hæmatin.}} + 2\,H_2O - Fe = \underbrace{C_{32}\ H_{36}\ N_4\ O_6}_{\text{Bilirubin.}}$$

It follows from this, according to *Neneki* and *Sieber*, that *bile pigment is formed from the colouring matter of the blood, in that its molecules lose iron and take up water.* *Latschenberger*[115] concludes, from experiments which he performed on animals, that bile pigment, or rather its antecedents, to which he gives the name of *Cholegloblin*, results from the decomposition of blood-colouring matter, a dark-coloured ferruginous pigment being formed at the same time. Choleglobin is elaborated both in the tissues and in the interior of cells.

It seems to the writer not unimportant to consider these views here, in anticipation of much that will have to be said later on concerning the colouring matters of the blood and of the bile, in their relations to one another.

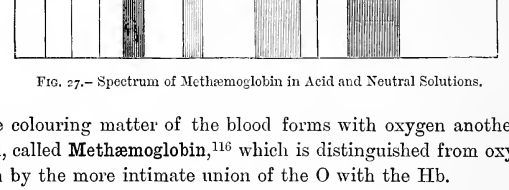


FIG. 27.– Spectrum of Methæmoglobin in Acid and Neutral Solutions.

The colouring matter of the blood forms with oxygen another compound, called **Methæmoglobin**,[116] which is distinguished from oxyhæmoglobin by the more intimate union of the O with the Hb.

The spectrum of methæmoglobin in acid and neutral solutions shows **four** absorption-bands (fig. 27), one well marked (between C and D), the other three in the yellow, green, and blue, being less easily seen. This spectrum, as already said, is indistinguishable from that of acid hæmatin in alcoholic solution. Any possibility of confounding these two bodies is, however, excluded by the fact that when *methæmoglobin* is acted upon with sulphide of ammonium, its spectrum gives place, first, to that of oxyhæmoglobin (fig. 22), and after a while to that of reduced hæmoglobin (fig. 23); whilst, on the other hand, a solution of *hæmatin* treated with ammonium sulphide yields a spectrum exhibiting two absorption-bands between D and E (fig. 25). In alkaline solution, the spectrum of methæmoglobin shows three bands, viz., a narrow one between C and D, but close to the latter, and two broader ones between D and E (*Jäderholm*).[117]

1. Blood-changes in Dyspnœa.—All conditions which interfere with the giving off of CO_2 and the absorption of O in the lungs are attended with certain characteristic changes in the blood.

The clinical symptoms of dyspnœa do not fall within our province. They result directly from the condition of the blood, which shows itself in the appearance of the patient. The arterial blood is laden with carbonic acid, and in consequence has a darker colour, and this imparts a bluish hue to the visible surfaces—cheeks, lips, nose, and finger-tips. Microscopical examination of the blood shows no changes of a special character. And further, in cases of the most severe dyspnœa, the blood is never so deficient in oxygen that its spectrum exhibits any considerable change, such as, for instance, the disappearance of the oxyhæmoglobin bands.

2. Blood-changes in Carbonic Oxide Poisoning.—In carbonic oxide poisoning the blood undergoes a change of colour which is appreciable by the naked eye. It becomes of a bright cherry-red, alike in the arteries and the veins. Spectrum analysis shows the most important

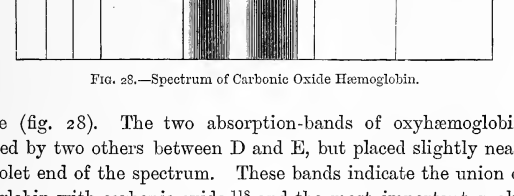


FIG. 28.—Spectrum of Carbonic Oxide Hæmoglobin.

change (fig. 28). The two absorption-bands of oxyhæmoglobin are replaced by two others between D and E, but placed slightly nearer to the violet end of the spectrum. These bands indicate the union of the hæmoglobin with carbonic oxide,[118] and the most important quality of this union is, that *these bands cannot, as in the case of oxyhæmoglobin, be made to disappear by the action of deoxidising agents* (ammonium sulphide, Stokes' fluid). [See Appendix VII.] Carbonic oxide hæmoglobin in the blood of the living subject may be recognised thus:—A few cubic centimetres of blood are taken from the patient by means of a cupping-glass, and mixed with water. Sulphide of ammonium is then added, and the solution is placed in a glass vessel with parallel sides, or, still better, the blood itself placed by means of Hénocque's apparatus before the slit of the spectroscope. If the specimen be one of blood poisoned with carbonic oxide, the two absorption-bands will remain in spite of the admixture with the reducing agent, sulphide of ammonium.

The presence of carbonic oxide in the blood can also be determined by the following chemical test:—To a quantity of blood mixed with

water a 10 per cent. solution of caustic soda is added. When the mixture is slowly warmed, a cinnabar-red colour appears. Under similar circumstances a solution containing oxyhæmoglobin will turn brownish-green (*Hoppe-Seyler, Otto*).[119]

A modification of this test has been suggested by *Salkowski*.[120] The blood to be examined is diluted with water to twenty times its bulk, and a like quantity of a solution of caustic soda (sp. gr. 1.34) is added. If the blood contains carbonic oxide, the fluid turns first white and cloudy, and presently a bright red; when allowed to stand, red flakes form and settle upon its surface. In the case of normal blood, when treated in this way, a dirty brown colouration results. *Kuniyosi Katayama's* method[121] is to add to the blood a little yellow sulphide of ammonium and dilute acetic acid. The presence of carbonic oxide will then be shown by the appearance of a beautiful red colour, whilst normal blood so treated turns grey or greenish-grey.

3. Blood-changes in Poisoning with Sulphuretted Hydrogen (*Hydrothionæmia*).—The investigations of *Hoppe-Seyler*[122] tend to the conclusion that hæmoglobin will enter into combination with H_2S, and form a substance which that author has called Sulphide of Methæmoglobin. It is, however, noteworthy that in the severest cases of poisoning with H_2S, the two absorption-bands in the spectrum of oxyhæmoglobin are never known to disappear. In such cases, the blood becomes peculiarly dark, and sometimes of a dull green tint. And it is further remarkable that the distinction between venous and arterial blood entirely disappears (*Lewin*).[123]

4. Blood-changes in Prussic Acid Poisoning.—*Preyer*[124] maintains that hydrocyanic acid forms a crystalline compound with hæmoglobin. However this may be, such a compound has not yet been found in the blood of men or animals poisoned with the drug. According to *Hoppe-Seyler*,[125] the union of hydrocyanic acid with hæmoglobin is of a very unstable character, the resulting body readily decomposing when crystallised or in presence of putrefaction.

5. Blood-changes in Poisoning with Chlorate of Potash.—*Marchand*[126] discovered that when potassic chlorate was taken in great quantities, the blood was profoundly altered, the most notable effect being the formation of a sepia-like decomposition product, which was afterwards shown to be identical with the methæmoglobin of Hoppe-Seyler, mentioned above. Large doses of chlorate of potash cause the production of methæmoglobin itself in the blood, especially of children. *Stokvis* and others[127] conclude from experiments upon rabbits that the exhibition of the salt is not attended with the formation of methæmoglobin in the blood of the living subject; while *Marchand* and *Cahn*[128] have, in fact, obtained this result in certain

animals, notably dogs. The opinion of the latter observers finds support in a clinical notice of *Lenhartz*, and also in a pathological observation recorded by *H. Hammer*.[129]

Chlorate of potash may be easily detected by spectrum analysis in fairly dilute solutions of hæmoglobin, and the spectrum of methæmoglobin, if present, will afford presumptive evidence of the poison. Methæmoglobin is produced also by the inhalation of nitrite of amyl (*Gamgee*) and the injection of sodium nitrite into the blood-vessels[130] (*Hoppe-Seyler*), as well as by kairin, thallin, hydrochinon, pyrocatechin, iodine, bromine, turpentine, æther, perosmic acid, permanganate of potash (*G. Hayem*) and antifebrin (*Fr. Müller*).[131] [The nitrites form a compound with its oxygen, more firmly fixed than that of the oxygen in oxyhæmoglobin. They consequently tend to stop internal respiration, but are more readily displaced by the products of asphyxia than is carbonic oxide hæmoglobin, and so again permit the aëration of the blood at the lungs.]

6. Poisoning with Nitrobenzol.—It has been asserted[132] that in dogs poisoned with nitrobenzol the spectroscope has shown blood-changes attributable to the presence of hæmatin. It would seem, then, that in any case of suspected poisoning by this means in the human subject the blood should be examined in this direction by the spectroscope.

7. Hæmoglobinæmia.[133]—By this term is meant the condition in which hæmoglobin is found dissolved in the blood. It is followed by *Hæmoglobinuria*, whenever the spleen and the liver are unequal to the task of eliminating the materials derived from the destruction of the red blood-corpuscles within the vessels.

The presence of free colouring matter in the blood may easily be determined thus:—A little of the blood, drawn from the patient by means of a cupping-glass, is placed immediately in a refrigerator, and allowed to remain there for twenty-four hours. If the blood is normal, perfectly clear yellowish-coloured serum will settle; whereas, if hæmoglobinæmia be established, there will be seen over the blood-clot a beautiful transparent ruby-red stratum. The spectroscope shows in the case of normal serum a feeble absorption-band in the blue part of the spectrum (at *F*), said to be due to lutein (*Thudichum*);[134] whilst with serum containing colouring matter it shows the two absorption-bands of oxyhæmoglobin.

8. Recognition of Changes in the Colouring-Matter of the Blood.—The changes in the character of the blood referred to above are chiefly to be estimated by means of the spectroscope. Very perfect little instruments for clinical use have been invented by Desaga of Heidelberg, Zeiss of Jena, and Hoffmann of Paris. Browning's spectroscope is also very suitable for the purpose.

To use one of these, artificial or day light is made to fall upon the slit of the instrument. The telescopic tube attached to the apparatus is focused until a spectrum is clearly defined, and if daylight be employed, the slit-like aperture is narrowed so as to bring Fraunhofer's lines clearly into view. The blood solution to be tested is then fixed between the aperture and the light. If the fluid be too concentrated, it must be diluted beforehand. If the light be artificial, whether from a lamp or some other source, it is well to place a little common salt or some other salt of sodium in the flame, in order to define the situation of the sodium line.

Very good results have been obtained in the investigation of blood spectra with the aid of *E. Hering's* [135] "*Lensless Spectroscope*," an instrument which especially commends itself to the practitioner by reason of its cheapness. It has been employed by the author together with Browning's pocket-spectroscope, and shown itself to be quite as serviceable. The lensless-spectroscope consists of two tubes, one sliding within the other, and of about $2\frac{1}{2}$ cm. diameter. Of these, the outer

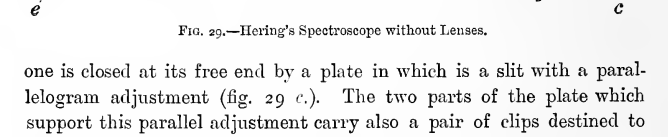


Fig. 29.—Hering's Spectroscope without Lenses.

one is closed at its free end by a plate in which is a slit with a parallelogram adjustment (fig. 29 *c*.). The two parts of the plate which support this parallel adjustment carry also a pair of clips destined to hold in position a rectangular glass vessel or test-tube containing the fluid to be examined.[136]

The tubes are lined with black, and the inner one (*a*) is provided at (*f*) with a diaphragm to intercept reflected light. At that end of the inner tube which is turned towards the observer, a prism (*d*) is fixed in such a position that the spectrum is formed in a plane at right angles to the proximal end of the tube, which is oblique, not vertical, in section. In using the instrument, the eye must be directed at right angles to this section, and not in the long axis of the tube. The tubes must also be adjusted in such a manner that the spectrum is well defined and accurately rectangular.

When this is done (by manipulation of the two tubes), a small but very clear spectrum is obtained, in which the yellow is little developed, but which very plainly exhibits absorption-bands such as those of oxyhæmoglobin and urobilin. The instrument serves admirably for the investigation of these bodies in blood and urine.

Hénocque's apparatus is the most appropriate for the examination of undiluted blood.*

2. Proteids of the Blood.—The proteids of the blood are **diminished** in all cases in which the total quantity of that fluid is greatly lessened—temporarily, therefore, in hæmorrhages of all kinds. A permanent diminution of proteids occurs under such conditions as disturb unfavourably the balance of waste and repair, whether of the blood itself or of its contained albumin. Thus, in all diseases which are attended with long-continued and excessive destruction of proteids in the system, these bodies are found to be proportionally wanting in the blood. It should be remarked, however, that such processes must be long continued before this effect is reached, especially where the digestive functions remain unimpaired. As a rule, a diminution of proteids goes hand in hand with an unduly watery state of the blood (*hydræmia*).

Hoppe-Seyler's[137] method is, perhaps, the best for determining the proportion of proteids in the blood; but we shall not describe it here, because it is very complicated, and its results are not altogether reliable.

The occurrence of an absolute **increase** of proteids has not yet been established on satisfactory evidence. A relative increase is known to happen in such diseases as are attended with the withdrawal of water from the system more rapidly than it can be supplied, as in cholera and acute diarrhœa.

In pneumonia and erysipelas there is an increase of fibrin. *Hoppe-Seyler*[138] has devised a method for the estimation of the quantity of fibrin in the blood, which is at once simple and adapted to clinical purposes. It may be described as follows:—A beaker of about 80 cc. capacity is provided with an india-rubber cap, perforated in the middle by a close-fitting rod of whalebone. These are dried and weighed. Next, 30–40 cc. of blood, taken from the body of the patient with a cupping-glass, are placed in the beaker, which is immediately covered by the india-rubber cap and its whalebone rod. The blood is then defibrinated by beating it up with the whalebone rod, allowed to cool, and weighed. The cover is removed, and the beaker filled with water and beaten up again. The fibrin is allowed to settle, washed with a solution of salt, and placed upon a filter whose weight is known. Here it is again washed with water until the fibrin is almost free from colouring matter. It is next boiled with alcohol (to dissolve fat, lecithin, and cholesterin), dried at 110°–120° C., then cooled, and weighed over sulphuric acid.

E. Ludwig[139] and the author have found peptones present in great

* This instrument can be had of *Rothe*, of Prague, for five florins.

quantity in the blood in leukæmia. To determine the presence of peptones in the blood, it is necessary first to remove the other proteids by the action of metallic oxides, and then to proceed in the manner indicated in the chapter on *Urine*.

3. Urea.—Urea occurs only in traces in healthy blood (*J. Picard*).[140] The following method will serve to detect its presence:—Blood is diluted with 3–4 times its volume of alcohol, the mixture allowed to stand for twenty-four hours, and filtered. The precipitate is washed on the filter repeatedly with more alcohol, the filtrates are mixed, and the alcohol distilled off. The residue is treated with nitric acid, and the resulting crystalline pulp allowed to stand for some hours, when the crystal masses which have formed are pressed between folds of blotting-paper, dissolved in water, and treated with carbonate of baryta until carbonic acid ceases to form, and dried on a water-bath; the dry residue is then extracted with boiling absolute alcohol. On evaporation, the urea remains in long slender prismatic crystals belonging to the rhombic system. If enough blood has been taken (at least 200–300 cc.), or if the blood happens to contain urea in large quantities, the following tests may be performed with the resulting crystals:—

1. Place some dissolved in a drop of water upon a slide, add a drop or two of moderately strong pure nitric acid, and apply a cover-glass. When looked at through the microscope, the characteristic hexagonal plates of nitrate of urea will be seen.

2. To a somewhat saturated solution of the crystals add a little metallic mercury and a drop of nitric acid, and heat; gas (CO_2 and N) is rapidly evolved.

3. Heat the dried crystals in a test-tube, add a trace of caustic soda and a drop of dilute solution of sulphate of copper. A violet colour (biuret) indicates the presence of urea.

4. Over a crystal of urea pour a drop of fairly concentrated watery solution of furfurol, and add immediately a drop of hydrochloric acid of 1.10 sp. gr. A play of colours takes place from yellow through green and blue to purple-red (*Schiff*).[141]

This reaction does not occur with uric acid, but is yielded by allantoin, though less promptly and clearly than by urea. It is given, moreover, by a number of other bodies.

When the above method * fails to exhibit the presence of urea—as, indeed, usually happens in testing blood, on account of the very small quantity of that body which it contains—resort must be had to the more accurate process of *Hoppe-Seyler*,[142] which can also be employed

* The method has been described here because it serves well for the purpose of examining the secretions and excretions generally for urea.

whenever a quantitative analysis of urea is attempted. *Von Schröder's* method[143] is, perhaps, the most delicate of all, but, on account of its minute details, it is hardly applicable to clinical purposes.

Urea is found in increased quantity in the blood whenever its elimination is interfered with, either by disease of the kidneys or obstruction of the urinary passages.

V. Schröder has shown that the formation of urea probably takes place in the liver.

4. Uric Acid.—*Garrod* found uric acid to the amount of 0.025–0.175 in a thousand in the blood of persons suffering from gout. It must be observed, however, that his method of testing for this substance was far from reliable.[144]

He took about 30–35 grms. of blood and allowed it to coagulate. Ten cc. of the serum were then mixed with a dilute acetic acid solution in the proportion of 1 : 10, and a delicate thread was placed in the fluid. When the blood contained not less than 0.025 per 1000 uric acid, it was found that, after twenty-four to forty-eight hours, the thread was covered with uric acid crystals.

To detect uric acid in the blood, the latter is diluted with water, and treated with acetate of soda and a solution of ferric chloride to 5–10 times its volume, the mixture neutralised with caustic potash, and the clear filtrate tested for the presence of uric acid by *Ludwig's* method (*Abeles*).[145] (See chapter on *Urine*.) The resulting crystals are washed first in cold water and subsequently with alcohol.[146] Then :—

1. Examine some under the microscope. The characteristic whetstone forms, and sometimes the rhombic tables, of uric acid crystals are seen (figs. 91 and 92).

2. Heat a small quantity of the crystals with nitric acid in a porcelain dish ; evaporate, and to that part of the mass which is reddish in colour add a trace of ammonia, when the colour will change to purple. If to another part of the mass a little caustic potash be added, a violet colour is seen (*murexide test*).

The proportion of uric acid in the blood may be estimated with some degree of accuracy by *Ludwig's* method, regard being had to the quantity of blood taken. The conditions which favour the appearance of uric acid in the blood are not yet satisfactorily known. It is a normal constituent of that fluid (*Abeles*). Its quantity is apparently increased in gout, and especially during the acute attack (*Salomon*).[147] On the other hand, it is found only in traces in chronic nephritis and acute articular rheumatism.

5. Sugar (*Melithæmia*).—In health the blood contains a minute quantity of sugar. To detect its presence there, the blood must first be freed from proteids, and for this purpose the old method of *Claude*

Bernard[148] is the best. This consists in adding to it its own weight of crystalline sodic sulphate, boiling, and filtering. [See Appendix VIII.] The filtrate is free from proteids, and may be tested for sugar thus :—

1. *Moore's test* will serve where sugar exists in any quantity. (See chapter on the *Urine*.)

2. *Trommer's test*. (See chapter on the *Urine*.)

3. *The phenyl-hydrazin hydrochloride test* is the best for detecting slight traces of sugar in the blood. It is conducted as follows (*v. Jaksch*) :[149]—

Add together two parts of phenyl-hydrazin hydrochloride and four parts of acetate of soda; add water and heat. Take 5 cc. of the proteid-free filtrate (which is practically a saturated saline solution), obtained by Claude Bernard's process, and while still warm add it to 5 cc. of the solution prepared as above. Place the mixture in a test-tube half filled with water, heat it for half-an-hour on a water-bath, and allow it to stand. Or a little of the phenyl-hydrazin salt and acetate of soda may be added in the dry state to the warm proteid-free filtrate, and the process conducted as described above. After it has cooled, when examined under the microscope, it is seen to contain separately and in clusters the characteristic yellow crystals of phenyl-glucosazon scattered amongst colourless crystals of sulphate of soda. (See chapter on the *Urine*.)

To determine the percentage of sugar in the blood, *Fehling's* fluid may be employed (the blood having been previously freed from proteids) in the manner afterwards to be recommended for testing for sugar in the urine, and the polarimetric test * may be applied. It seldom happens, however, that the filtrate contains sufficient sugar to be appreciable with the polarimeters at present in use. *Lippich's* instrument is the most sensitive, and gives the best results in this connection.

In diabetes large quantities of grape-sugar are found in the blood. *Hoppe-Seyler*[150] describes a case in which it reached as high as 0.9 per cent. The researches of *Freund*[151] would make it appear that a deoxidising substance—presumably sugar—exists in considerable quantity in the blood in cases of carcinoma. [This statement, however, needs further confirmation.] The blood of tubercular patients is said by the same observers to contain cellulose.[152]

For the detection of sugar and the carbohydrates generally in the blood, the processes of *Baumann* and *Udransky*[153] may be employed with advantage. These are based upon the fact that the carbohydrates are precipitated from their watery solutions by the addition of benzoyl chloride and caustic potash, forming insoluble compounds. This combination of the carbohydrates with benzoyl chloride may easily be

* See chapter on the *Urine*.

recognised by its characteristic colour-reaction (furfurol) when treated with sulphuric acid.

6. Organic Acids in the Blood (*Lipacidæmia*).—Traces of volatile fatty acids are sometimes present in the blood. The author has frequently met with them. For their detection 20–30 grms. of blood are taken from the patient by means of a cupping-glass, an equal weight of sulphate of soda added, and the whole boiled and filtered. The filtrate is evaporated to dryness, and the residue extracted with absolute alcohol. The alcoholic extract in a large number of cases contained no fatty acids, but, on the other hand, these occurred in traces whenever looked for in fever and leukæmia, and occasionally in diabetes.[154]

Lactic acid is also sometimes met with. Normal venous blood has been said to contain 0.0079 per cent. of sarco-lactic acid (*Berlinerblau*). In regard to the latter and its tests, the reader may be referred to the statements of *Hoppe-Seyler* and *Berlinerblau*.[155]

7. Lipæmia.—The blood invariably contains small quantities of fat. While digestion is in progress it abounds in this substance normally; but a permanent excess of fat is also a phenomenon of certain morbid states. The blood in such cases is altered to the naked eye. It is turbid and usually paler than in health. Under the microscope a number of minute strongly-refracting globules are seen floating amongst the proper cellular elements. The white corpuscles also often contain fatty particles. If any doubt remains in a given case as to the nature of these particles, the addition of æther will settle the matter. If they are fatty, a drop of æther poured upon the slide will dissolve them and cause them to disappear.

Lipæmia has been met with in chronic alcoholism, chronic nephritis, and severe cases of diabetes. It also occurs in wounds of the medullary cavity of bones (embolic lipæmia) when fluid fat passes into the blood.

8. Cholæmia.—By this term is meant the condition in which the constituents of the bile are found in the blood. In this connection the biliary acids and colouring matters (bilirubin) are the points of chief interest to the physician. And of these, again, the biliary acids must probably be regarded as the real toxic agents, involving great possibilities of mischief, leading to the disintegration of the red corpuscles, and, as a consequence, to hæmoglobinæmia, disturbing the innervation of the heart, and slowing the pulse. Even where such symptoms are present, however, the quantity of biliary acids in the blood is always very small—so small at times as to escape detection by the chemical method presently to be detailed. This method, nevertheless, deserves to be described, since it will serve where the biliary acids exist in comparative abundance in the blood, and in all cases where

the secretions are tested for bile. We shall, therefore, introduce it here :—

The blood[156] to be examined must first be freed from proteids by precipitation with alcohol, or boiling it after dilution and filtering. The proteid-free filtrate is treated with acetate of lead and ammonia. The biliary acids combine with the lead and are precipitated as lead salts. The precipitate is washed with water on a filter, boiled in alcohol, and filtered. Carbonate of soda is added to decompose the lead salt. The solution is again filtered, evaporated to dryness, and the residue extracted by boiling with absolute alcohol. On evaporation, the bile salts will crystallise out, or a dull amorphous substance may remain, from which the crystals can be derived by the addition of æther. The amorphous substance itself may be tested for biliary acids by *Pettenkofer's*[157] method. This test depends upon the reaction of cholalic acid in presence of cane-sugar and sulphuric acid. To apply it, dissolve some of the crystalline or amorphous residue, obtained as above, in water; add two-thirds its bulk of sulphuric acid slowly, so that the temperature may not be raised above 65°. To the mixture now add a few drops of a solution of cane-sugar (1 to 5 of water), and a beautiful violet colour indicates the presence of biliary acids. According to *Mylius*,[158] this test depends on the formation of furfurol from grape-sugar, which then gives a play of colour with the bile acids. The reaction may also, therefore, be well displayed with furfurol.

Mackay's[159] physiological test may sometimes serve for the detection of these substances in the blood. It depends upon the action of bile acids, as observed in experiments, on the atropinised frog's heart.

If a known quantity of blood be taken, the *proportion* of bile acids in the blood may be determined in the manner described above. Efforts have been made, unsuccessfully, to base an analytical test upon the polarisation phenomena of biliary acids.

Bilirubin may be recognised by testing the serum obtained from blood which has been allowed to coagulate on ice by any of the methods described later on (*vide* chapter on the *Urine*). *Huppert's* test is the best for this purpose.

The same thing may be done more simply in a manner which the author has recently adopted. Blood is taken from the patient with a cupping-glass, placed in a test-tube in a refrigerator, and when coagulation has taken place the serum is withdrawn by means of a pipette, and put into another test-tube. It is then shaken into froth. If bile pigment be present, this froth is yellow. In all other cases (as, for instance, in hæmoglobinæmia, where the serum itself is tinted) the froth is quite colourless.[160] Moreover, if more of the serum be now taken, and left for three or four hours in a warm chamber at 35° C.,

the development of an intense green colour will mark the formation of biliverdin. A mere trace of bile pigment will cause this green colour to appear, whereas normal serum remains unchanged.

The microscopical examination of jaundiced blood generally shows nothing unusual. According to *Silbermann*,[161] this condition in newly born infants is marked by the following changes:—The red corpuscles are more or less disintegrated; they are often pale, or exhibit only round the pale centre of the corpuscle a ring of hæmoglobin of normal tint. Blood-plates, macro- and micro-cytes, and poikilocytes are to be seen, together with nucleated red corpuscles and corpuscle-holding cells. In a case which came under his own observation, however, the author failed to find such appearances. They are not to be regarded as constant in jaundice.

9. Uræmia.[162]—When urinary products accumulate in the blood, the condition is termed *Uræmia*. The retention of these products is marked by certain well-defined phenomena, even though we are not at present able to refer them to the action of any one substance in particular. The assumption that the poisonous material is urea, or carbonate of ammonia resulting from its decomposition, has been disproved. It is now believed that the symptoms of uræmia are due in general terms to the excessive accumulation of fixed products in the blood. The interesting researches of *Bouchard*[163] go far to show that they may be referred to the toxic effects of certain bodies resembling alkaloids (ptomaines) normally existing in the urine. Uræmic blood shows an increased quantity of urea and extractives. In a number of cases reported by *Horbazewski*, no increase in salts was noted, even in the salts of potash. The author himself in several instances has observed that the alkalinity of the blood was greatly less than normal.[164] There are no other characteristic changes to be noticed as occurring in the blood in uræmia.

10. Ammoniæmia.—Of this condition very little is yet known. From observations hitherto made, it would appear that the poisonous phenomena are due to the action of some body—probably an alkaloid—introduced into the system by absorption from the diseased bladder. It would be essential in such cases to examine the blood for ptomaines.

11. Acetonæmia.—This term is applied to a condition in which the blood is surcharged with acetone.[165] *Deichmüller* and the author have succeeded, by extracting the blood with æther and subsequent distillation, in separating from it a substance which gives the reactions of acetone. In many morbid states, and especially in fevers, it is found in considerable quantity.

12. Changes in the Salts of the Blood.—The blood contains about one-half per cent. of chloride of sodium,[166] and this quantity

remains constant, whether much salt be taken with the food or not. Moreover, *Schenk*[167] has shown that in fevers, as, *e.g.*, pneumonia, where the chlorides disappear from the urine, the proportion of salt in the blood is not notably altered.

In rickets and osteomalacia the salts are diminished.

The blood of tubercular patients, according to *Freund*,[168] is relatively deficient in sodium salts and phosphates, whilst at the same time the salts of potash are increased.

The tests and methods for the qualitative and quantitative analysis of the salts of the blood are to be found in the various text-books of physiology and physiological chemistry.[169]

CHAPTER II.

THE BUCCAL SECRETION.

The saliva is a mixed secretion, derived in part from the mucous glands within the mouth, and partly from the parotid, submaxillary, and sublingual glands, which open by ducts within that cavity. Any disproportionate activity, whether in health or disease, of one or other of these glands will be attended with a corresponding modification of the physical and chemical characters of the saliva.[1]

I. NAKED-EYE APPEARANCES OF THE SALIVA.—The saliva, when freshly taken from the mouth, is a colourless or light blue fluid, usually somewhat thick and stringy. When allowed to stand for some time, it settles into two layers, of which the lower one is quite cloudy and turbid, and contains in the greatest abundance the morphological constituents presently to be described.

The reaction is distinctly alkaline.

II. MICROSCOPICAL APPEARANCES.—The saliva when examined with the microscope is seen to contain certain morphological elements in varying proportions. These are:—

1. Salivary Corpuscles.—These bodies resemble white blood corpuscles, but are larger, and their protoplasm is usually very granular.

2. Red Blood-Corpuscles.—These are seldom met with, and when they occur are readily recognisable.

3. Epithelium.—Usually in the form of large irregular squamous cells, derived from the mucous membrane of the mouth and tongue. The quantity of epithelium to be found in the saliva varies greatly in health; and the cells exhibit much difference in shape, according as they come from the superficial or the deeper layers of the mucous membrane. They are, however, easily known by their polygonal shape and relatively large size.

4. Fungi.—Mould and yeast fungi are very seldom seen in the saliva in health; when they occur, it is as an accidental constituent, probably

introduced with the food. In disease, however, their presence is frequent. Fission-fungi, on the other hand, are met with in great number and variety in healthy saliva. There are to be seen, thickly scattered through the secretion, smaller or larger colonies of micrococci, of which some possess the property of staining reddish in a solution of iodine and iodide of potassium; bacilli, too, of varying size, which take a bluish red colour with the same reagent. There is also an organism called the Spirochæte buccalis, which occurs as extremely mobile spiral threads, very closely resembling the spirillum of relapsing fever; from this it is distinguished chiefly by its greater breadth and by the smaller number of its coils. Forms resembling the comma bacillus are frequently found in the saliva (*Lewis*,[2] *Miller*[3]). They have been obtained in considerable numbers from the secretion (*Vignal*).[4] As many as twenty-one different micro-organisms have been separated by the ordinary methods (see Chapter X.), and cultivated on plates and

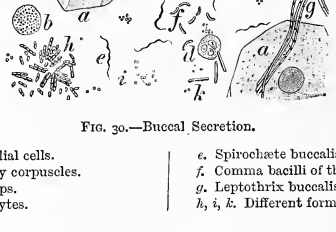


FIG. 30.—Buccal Secretion.

a. Epithelial cells.
b. Salivary corpuscles.
c. Fat drops.
d. Leucocytes.
e. Spirochæte buccalis.
f. Comma bacilli of the oral cavity.
g. Leptothrix buccalis.
h, *i*, *k*. Different forms of fungi.

by inoculation, and their behaviour in various food media observed. *Biondi*[5] has recently been engaged in such research. According to *W. D. Miller*,[6] the following list is a summary of the pathogenic fungi which have hitherto been found in the buccal cavity, part of these having also been isolated by cultivation methods:—Leptothrix buccalis, Vibrio buccalis, Spirochæte dentium, Micrococcus tetragenus, Micrococcus de la rage (*Pasteur*), Micrococcus of septicæmic sputum, the fungus designated 8 by Miller, being the bacillus of decaying teeth, Bacillus crassus sputigenus, Bacillus salivarius septicus, two pathogenic fission-fungi not yet cultivated (*Kreibohm*), Staphylococcus pyogenes, albus and aureus, and Salivarius pyogenes, Coccus salivarius septicus, and Bacillus septicus sputigenus.

Miller[7] has cultivated over fifty different fungi obtained from the mouth. In his opinion, caries of the teeth is due to the action of

several varieties, which he supposes to generate acids, or to have the power of destroying decalcified dentine.

To show Spirochæte buccalis, a drop of pure saliva should be examined as it is with a good oil-immersion lens, an Abbe's condenser, and a narrow diaphragm. It may be seen also in a preparation stained by *Günther's* process (see p. 29).

Under pathological conditions other pathogenic fungi are to be found in affections of the mouth; as, for instance, Oïdium albicans, Actinomyces, and the bacilli of tubercle and pneumonia. *Fränkel*[8] has obtained the bacillus of typhoid from the lingual glands in a case of death from that disease, and doubtless our acquaintance with such forms will extend with our knowledge.

III. CHEMICAL CONSTITUTION OF THE BUCCAL SECRETION.—This varies even in health with the activity of the different glands by which the fluids are secreted. There are to be found traces of albumin coagulable with heat, mucin, and occasionally sulphocyanide of potassium (CNSK). The saliva contains, further, a ferment which changes starch into sugar, and a trifling amount of salts. Oxygen, nitrogen, and carbonic acid gases have been obtained from parotid saliva (*Külz*).[9]

It is seldom that opportunity offers for a chemical examination of the saliva in disease. The quantity is then generally diminished rather than increased; and further, it is very difficult to obtain a pure secretion from the patient.

Ptyalism (*see below*) is the only morbid state in which a large quantity of pure saliva can be had. To examine the saliva, the patient should be made to rinse the mouth with water carefully after each meal, and the secretion collected for twenty-four hours in a clean vessel. The reaction may be tested with litmus paper and the specific gravity taken. It will be found to be alkaline, and of sp. gr. 1.002–1.006. A portion of the fluid may next be tested for albumin in the manner to be described in the chapter on *Urine*.

Another portion may be tested with solution of ferric chloride for sulphocyanides. Should such be present, a bright red colour appears, which does not disappear either with heat or on the addition of acid. If the red colour is not thus obtained, 100 cc. of the saliva should be concentrated on a water-bath and tested as before.

Sugar may be tested for in the manner recommended for its detection in blood, No. 3 (*v. supra*, p. 48).

The presence of diastatic ferment may be shown thus:—5 cc. of saliva are mixed with 50 cc. of starch solution, and placed in a warm chamber or in a water-bath heated to 40° C. When examined

after one hour, the fluid (which of course must have been tested beforehand to ascertain the absence of sugar) will give all the reactions of grape-sugar if amylolytic ferment be present.

Nitrites often occur in saliva. They may be detected by adding to a little of the fluid a mixture of starch paste, iodide of potassium, and dilute sulphuric acid, when, if nitrites be present, an intense blue colour will be seen. A very useful test for nitrites has been suggested by *Griess*.[10] To a specimen of saliva diluted with five times its bulk of water, a few drops of sulphuric acid are added, and then metadiamido benzol which melts at 63° C. The appearance of an intense yellow colour shows the presence of nitrites. (See Appendix IX.)

IV. CONSTITUTION OF MORBID SALIVA IN GENERAL.—The quantity of saliva is **diminished during inflammation** of the salivary glands in febrile disorders and diabetes, and often also in nephritis. [In high fever no saliva is secreted. That of moderate fever is thick and scanty, and usually acid, and with the rise of temperature its diastatic action is lessened.] It is **increased** in inflammations of the mouth, by the action of certain poisons—as, *e.g.*, pilocarpin and mercury —[in trigeminal neuralgia] and sometimes by the irritation of carious teeth. The excessive secretion which attends poisoning by acids and alkalies is rather due to irritation of the ducts than to any specific action on the salivary glands. A long-continued flow of saliva will sometimes occur without its being possible to ascribe it to any of the causes mentioned. In such cases probably the disturbance is due to some obscure changes in the innervation of the glands. Salivation has occasionally been recorded as occurring in pregnancy (*Schramm*).[11]

These are the rare cases (referred to above) which afford a favourable opportunity for chemical analysis of the saliva.

In a case of ptyalism which the author observed, analysis of the saliva showed that it contained 995.2 grms. of water and 4.8 grms. of solids. Its reaction was alkaline. It held a small quantity of *mucin*, traces of serum-albumin, and some sulphocyanides. The iodide of starch test showed the absence of nitrites; and no sugar was detected by phenyl hydrazin or other reagents (*Salkowski*).[12]

Certain diseases are attended with notable qualitative changes in the saliva. Thus in nephritis considerable quantities of urea have been found in it by *Wright*, *Picard*, *Rabuteau*,[13] and *Fleischer*.[14] For its detection Fleischer employs the following method:—An alcoholic extract of the saliva is made and filtered; the filtrate evaporated, and the residue dissolved in amyl-alcohol. Crystals of urea remain after evaporation, and may be recognised by any of the tests described at p. 46. *Boucheron*[15] found uric acid in the saliva of uræmic patients by employing the murexide test (p. 47).

Bile pigment and sugar have not yet been found in saliva. Even in that of diabetic patients there seems to be no sugar. In three cases of diabetes the author has carefully tested the secretion after the injection of pilocarpin, by means of the phenyl-hydrazin test, but in each case without any result.

Certain drugs, and amongst them iodide and bromide of potassium, are readily detected in the saliva soon after they have been taken into the system. (See chapter on the *Urine* for method of investigating this body.)

[**V. THE SULPHOCYANIDE OF THE SALIVA.**—The origin and purpose of this salt in the economy have long been a subject of speculation to physiologists. The researches of *Dr. S. Fenwick*[16] have invested the matter with a new interest, and their results go to prove that we have in the variations of its quantity a valuable index to certain states of the system. He has collected the records of a large number of cases in which the saliva was examined, and the quantity of sulphocyanide carefully compared. This was done by noting the colour produced by adding to the secretion a certain quantity of a standard solution of perchloride of iron. For the purposes of comparison, the tint so obtained with a mixture of the secretions from many healthy persons is taken to indicate the normal amount of the salt, and a scale of colours is prepared by evaporating and diluting the fluid to certain proportions, and copying the tints in each case.

As a result of his observations, Dr. Fenwick concludes that the sulphocyanide of the saliva is a measure of the functional activity of the nutritive organs, and that it is increased in general whenever an unusual demand is made on them by the necessities of the system, provided those organs are capable of responding to the call,—in the early stages of acute inflammation, of cancer and phthisis, in acute congestion of the liver from alcohol and over-feeding, in acute rheumatism, gout, and urticaria, and in convalescence from typhoid and similar diseases. The quantity is diminished in all conditions where the nutritive organs are unable to supply the requirements of the system, in the later stages of phthisis and malignant disease, in long-continued diarrhœa and dysentery, in jaundice from obstruction, in lead-poisoning, and in ascites and similar conditions impeding the portal circulation, and where the assimilation of food is imperfectly performed. Where, in connection with articular rheumatism, the sulphocyanide is greatly in excess, a tedious recovery is to be expected, and frequent relapses may be feared.

Dr. Fenwick believes that the sulphocyanide is derived from the decomposition of biliary compounds (? taurocholate of soda).

A more accurate method of ascertaining its amount is to collect the saliva secreted during five minutes, add the tincture of perchloride of iron in the proportion of one drop to a drachm, and filter. The colour obtained in this way may then be compared with solutions of sulphocyanide of iron carefully graduated. The bottles containing the tests,

and the solutions to be compared with them, should be of exactly equal size. The flat-sided vessels or hæmatinometers used for the spectroscopic examination of blood will serve well.]

VI.—THE SALIVA IN SPECIAL DISEASES.

1. Catarrhal Stomatitis.—This affection is regularly attended with a much-increased flow of saliva, which when examined microscopically is found to contain an excess of epithelium and many leucocytes, but is otherwise unaltered.[17] [Its reaction is acid.]

2. Other Forms of Stomatitis (*Mercurial, Scorbutic, &c.*).—The saliva is fœtid, dark brown in colour, and strongly alkaline. It is loaded with tissue *débris*, leucocytes, broken-down red blood-corpuscles, and various forms of fungi in abundance.

3. Thrush.—The presence of this fungus in the mouth demands a more detailed notice.[18] It occurs most frequently in children, but is

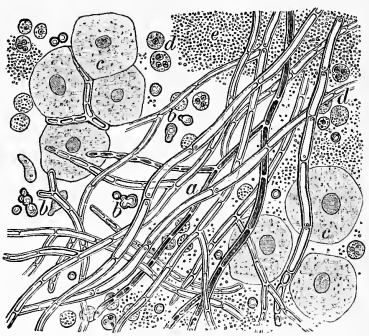


Fig. 31.—*a*. Thrush fungus; *b*. Conidia; *c*. Epithelial cells; *d*. Leucocytes; *e*. Débris.

common also in adults,[19] especially in association with tuberculosis. It used to be taught that the saliva of thrush is always acid; but it is still a matter of doubt whether the acidity is not due rather to the presence of other micro-organisms than to the action of the thrush fungus. *Kehrer* has shown that the latter parasite will thrive well in a medium where no free acid exists, as in lactate of sodium or potassium. The outset of the disease is marked by the formation of white patches on the mucous membrane, and when examined microscopically, these patches are seen to enclose sharp-bordered oval cells, each having one or two nuclei. The cells are disposed in groups of two or three. After the lapse of some days the patches run together, and form a membrane which may cover the entire mucous surface of the mouth, and even line the fauces and œsophagus.

The membranes are at first firmly adherent, but later on loosen, and may then be easily detached. When examined microscopically, they are seen to consist of epithelial cells, leucocytes, and *débris*, amongst which the parasite appears as branching ribbon-like forms composed of long segments. Each segment usually contains two strongly refractive nuclei embedded in a clear substance, one at either end. The segments vary in length, and grow shorter towards the extremities of the parasite. They are for the most part homogeneous, but occasionally finely granular. There are also to be seen the oval bodies figured above, which are thought to be the spores (conidia) of the fungus.

There is still much dispute as to the place of the thrush fungus in the vegetable kingdom. *Rees*[20] refers it to the yeast fungi; *Grawitz*[21] supposes it to be identical with the fungus studied by Cienkowsky; and *Plaut*[22] opposes this view, regarding it (with both the above-named authors, and also *Baginsky*[23] and *Klemperer*[24]) as a yeast fungus.[25] According to the more recent investigations of *Plaut*[26] the thrush fungus is identical with the widely distributed Monilia candida.

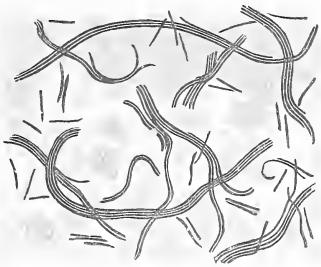


Fig. 32.—Leptothrix buccalis.

The fungus can be easily examined by placing part of the loose membrane with a little glycerine under the microscope.

When pus containing Actinomyces has been discharged into the mouth, the micro-organism can be found in the saliva. For its recognition, see the chapter on *Pus*.

Fischer and *Hauser*[27] have repeatedly found sarcinæ in the buccal mucus of wasting diseases.

VII. DEPOSIT ON THE TEETH.—If a little of the tartar be removed from the teeth with a spatula and examined, it will be seen to abound mainly in micro-organisms. These comprise:—

1. *Spirochæte buccalis* (mentioned above), in small numbers.

2. *Leptothrix buccalis.*—Long bacilli, usually segmented and arranged in large ribbon-like bundles. They stain bluish-red in iodine-potas-

sic-iodide solution (fig. 32). They are thought by *Miller*[28] and *Zopf*[29] to be, as stated above, with other micro-organisms, the cause of caries of the teeth. Mixed up with the masses of leptothrix are usually to be seen shorter bacilli, which do *not* stain in the iodine solution.

3. Various forms of micrococci, occurring both separately and in colonies.

4. A large number of leucocytes and epithelial cells, usually showing advanced fatty degeneration (fig. 30).

VIII. COATING OF THE TONGUE.—1. In severe infectious diseases the tongue is coated with a *brownish* fur, which consists partly of the remains of food and partly of dried blood. Microscopical examination of the coating removed from the tongue exhibits, in such a case, a profusion of epithelial cells and hosts of fungi of various forms. In addition to these, there is a multitude of dark cellular bodies, derived doubtless from the corneous and exfoliated epithelium of the part (*Bizzozero*).

Schech,[30] again, has called attention to the occurrence of a *black* fur, which is probably conditioned by the formation of pigmented papillæ on the tongue.

2. The tongue of infants is normally coated with a *white* fur, and a similar appearance is found in adults when the stomach is deranged. Microscopical examination shows epithelium, a few salivary corpuscles, and very many fungi.

[*Dr. Dickinson*[31] has recently investigated the nature and significance of the various morbid coatings of the tongue. He believes that a just conclusion cannot be arrived at from the inspection of material scraped from the surface, and his method was to obtain postmortem sections through the substance of the tongue, associating the microscopical with the naked-eye appearances during life. Thus it is seen that the different varieties of coating distinguished as "stippled," "coated," "plastered," "furred," and "encrusted," are all alike derived from excess and alteration of the epithelial elements of the tongue; the change, where most profound, extending first between the papillæ, and then deeper, with hypernucleation of the deep cells of the corium and the diapedesis of leucocytes. The presence of non-pathogenic fungi is common to all, and may be regarded as accidental; even the thrush fungus occurs independently of the grosser changes to which it commonly gives rise. The conditions of dryness and moisture greatly modify both the character of the coat and its significance as a symptom. The colour of the encrusted brown variety is due to dryness alone, while at the same time it must be mentioned that a profusion of micro-organisms is especially associated with this form. Clinically

the fact of most importance is the thickness and exuberance of the coating, and a comparison of instances has shown that such redundancy is especially connected with pyrexia.]

IX. COATING OF THE TONSILS.—The examination of morbid deposits upon the tonsils is sometimes of the utmost importance in diagnosis.

1. Croupous and Diphtheritic Tonsillitis. — The membrane covering the tonsils both in croup and diphtheria is composed of glistening homogeneous fibrin, disposed in the form of a network, of which the meshes vary in shape and size, and enclose epithelial cells, blood- and pus-corpuscles, and micro-organisms of every description. The differences between croupous and diphtheritic membrane cannot be distinguished by microscopic examination alone, as was formerly taught. In both cases whitish layers are found on the tonsils. It should be mentioned, however, that *E. Wagner* discriminates between croupous and diphtheritic tonsillitis, in that the removal of the membrane in the former affection leaves the underlying tissues simply hyperæmic and infiltrated with serum, while in the diphtheritic form a hæmorrhagic or even sero-purulent infiltration remains.

Hitherto even the newer bacteriological methods of investigation have not thrown much light on the subject. The researches of *Löffler*,[32] and the discovery by that author and *Klebs* of supposed distinctive bacilli, gave rise to the hope that we had attained to an easy method for the diagnosis of diphtheria; but more recent investigations, and especially those of *G. v. Hoffmann-Wellenhof*,[33] have shown that the difficulty is not yet solved.

Peters[34] discovered gregarina-like bodies (*Coccidium oviforme;* see chapter on the *Fæces*) in diphtheritic membrane stained with alum carmine and picric acid. This fact is noteworthy, but further observation must show in what relation these forms stand to the disease in man.

2. Pharyngomycosis Leptothricia.—A special interest has recently come to be attached to the nature of the plugs which block the tonsillar crypts. They are found in almost every healthy person, and consist of epithelial cells and of long segmented fungi, which stain bluish-red with the iodine-potassic-iodide solution. In certain conditions these micro-organisms extend outside the follicles, and cover the surface of the tonsils with patches of varying size. They then give rise to subjective symptoms, and their appearance may be mistaken for a commencing attack of croupous or diphtheritic tonsillitis. They may be readily recognised under the microscope by their reaction with the iodine solution, as mentioned above, and the course of the disease will afford a further indication of their nature (*Th. Hering*).[35] *O. Chiari*[36]

is of opinion that this affection is not one *sui generis*, but should merely be regarded as a modification of *Angina follicularis*, in which such products are always found.

One or two minutes in the iodine solution suffice to develop the bluish-red colour in a specimen containing leptothrix. This colour disappears in from twenty-four to seventy-two hours.

Dr. O. Chiari has called the attention of the author to the fact that yellowish plugs which do not contain leptothrix are often to be found in the crypts. In a very hard concretion from the tonsils, found on chemical examination to consist of carbonates and silicates, the author met with splendid specimens of leptothrix.

CHAPTER III.

THE NASAL SECRETION.

I. NAKED-EYE AND MICROSCOPICAL CHARACTERS—CHEMICAL CONSTITUTION.—Considering the great quantity of glandular tissue with which the nasal passages are furnished, the secretion in health is remarkably scanty.

Normal nasal mucus exhibits microscopically squamous and ciliated epithelium in abundance, isolated leucocytes, and an enormous profusion of fungi.

E. Weibel[1] has described a curved bacillus obtained from the nasal secretion of healthy persons, which, when cultivated in nutrient gela-

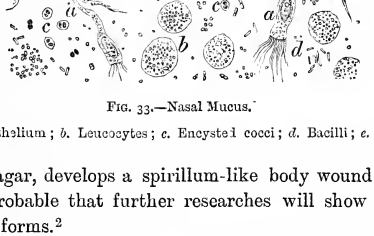


FIG. 33.—Nasal Mucus.

a. Ciliated epithelium; *b*. Leucocytes; *c*. Encysted cocci; *d*. Bacilli; *e*. Micrococci.

tine and agar-agar, develops a spirillum-like body wound into several coils. It is probable that further researches will show the presence of many other forms.[2]

The normal nasal secretion is a thick fluid, faintly odorous, and of an alkaline reaction. It abounds in mucin, but otherwise nothing definite is known about its chemical constitution.

II. THE SECRETION IN AFFECTIONS OF THE NASAL CAVITIES.—At the outset of an attack of acute nasal catarrh, the mucous membrane is generally dry and much injected, and the secretion lessened in quantity. Later on, however, there is a copious discharge of a thin

alkaline fluid, and this, when examined under the microscope, is seen to consist of a great number of epithelial cells and fungi.

Where suppuration is in progress within the nose, the secretion partakes of the character of pus, and is seen microscopically to consist almost entirely of pus cells. Occasionally, as in cases of wounds perforating the cranium and in brain tumours, cerebro-spinal fluid may be discharged through the nose. *Nothnagel*[3] has reported a very interesting case of this kind. Under such circumstances, chemical analysis showing the absence of albumin and the presence of sugar or, at least, of a reducing substance, will determine the diagnosis. The importance of the evidence so obtained in connection with cerebral tumours is evident.

It is very necessary, in all cases of ulceration of the mucous membrane of the nose, to look for certain of the pathogenic fungi already known to us.

Thus, if it be a question whether a particular ulcer is tubercular or not, a little of the discharge may be removed with the help of the nasal speculum on a carefully sterilised platinum spatula, and examined for tubercle bacillus in the manner indicated at p. 75.

Again, the discovery of the characteristic bacillus of glanders in such a discharge will obviously determine the diagnosis of that disease. This bacillus may be sought for in the same manner as in the examination of blood (p. 32). If this be not enough, fungi may be cultivated on *Koch's* plan (see Chapter X.); or, finally, in case of doubt, they may be propagated in one of the lower animals, when a definite conclusion will be arrived at.

E. Fränkel[4] and *Hajek*[5] observed various forms of fungi invariably present in the discharge of the chronic ulcerative processes known as ozæna. *Löwenberg*,[6] on the other hand, found that one large species of diplococcus was almost the only form present in such discharge, and he regards it as characteristic of ozæna.

Tost[7] and *Löwenberg*[8] have shown that bodies resembling the pneumonia coccus occur in the nasal secretion (fig. 33, *c*).

In a few cases, thrush fungus and vegetations have been found in the nose. Mould fungi in this situation are another rare manifestation (*Schubert*).[9] Ascarides and other entozoa are very seldom seen there. Dipteral larvæ are of most common occurrence (*B. Fränkel*).[10]

The *Charcot-Leyden* crystals found in blood and sputum have also been met with in the nasal secretion of an asthmatic patient; but their presence may probably be regarded as accidental.

Concretions (rhinoliths) occasionally form in the nasal cavities (*O. Chiari*,[11] *Seifert*[12]).

CHAPTER IV.

THE SPUTUM.

Under the term **expectoration** or **sputum**[1] are comprised all those substances which are removed from the air-passages by the mechanical effort of coughing or hawking. The sputum is, therefore, to be regarded as a mixture of many glandular excretions, to which may be added, under morbid conditions, the greatest variety of pathological products.

I. NAKED-EYE CHARACTERS OF THE SPUTUM.—The naked-eye appearance of the sputum will often afford valuable information; and that this may be as accurate and exhaustive as possible, it is well to collect the expectoration in glass vessels after Nothnagel's plan, and then to examine it as to its quantity, specific gravity, reaction, colour, smell, and tendency to stratification.

The **quantity of expectoration** discharged in twenty-four hours varies within broad limits. Sometimes it does not exceed a few cc.; in certain conditions, on the other hand, as, *e.g.*, when an empyema is discharging into the lung, as much as 800–1000 cc. may be expectorated in twenty-four hours.

H. Kossel[2] determines the **specific gravity** of the sputum in the following manner:—The sputum is placed in a flask stoppered to prevent the evaporation of the water, and gradually heated to 60° C. It is thus reduced to a thinly fluid state and is then placed in the pycnometer. The sp. gr. is seen to vary within very broad limits—for mucous sputum, 1.0043–1.0080; for purulent, 1.0155–1.0260; and for serous, 1.0375. The question of density has in no case any clinical interest.

The **reaction** of the sputum is always alkaline. In some diseases, as in abscess and gangrene of the lung, there is marked stratification of its parts (*vide* pp. 90 and 91).

The **colour** of the expectoration depends partly upon its microscopical, and partly upon its chemical character. When it consists entirely or chiefly of mucin and a few cells, it is whitish. Green sputa are usually purulent, but the presence of biliverdin or of pigment-forming bacteria may also impart this colour.

The **odour** of the sputum is for the most part not characteristic; but in putrid bronchitis and gangrene of the lung it has a particularly unpleasant and pungent smell.

For many purposes the admixture with water in a glass cylinder will facilitate the examination of the sputum. In this way, for instance, the nummular arrangement becomes apparent. In other cases, again, certain constituents, as, *e.g.*, spirals, fibrinous coagula, and shreds of tissue, may be made more evident on a dark surface, such as that of a polished black plate.

Although much knowledge of a disease may be derived from the naked-eye inspection of the sputa, it will never enable us to dispense with the aid of the microscope, by means of which alone we may diagnose certain affections—some forms of tuberculosis, for instance—with the utmost confidence.

II. MICROSCOPICAL EXAMINATION OF THE SPUTUM.

1. White Blood-Corpuscles.—These bodies are always to be seen in the sputum, commonly embedded in a viscid stringy substance. Many of these are of large size and granular, and enclose within them drops of fat and particles of pigment, such as carbon dust and masses of hæmatoidin (see fig. 34, *f*, *f'*). In cases where an abscess has discharged into the lung, and in purulent bronchitis such as is met with in connection with emphysema, the sputum may consist entirely of leucocytes.

2. Red Blood-Corpuscles.—These are also to be found in almost all sputa, and their presence in small number is without any special significance. In persons who smoke a great deal, or spend much of their time in an atmosphere of tobacco-smoke, the sputa are apt to be streaked with blood in the morning. This blood, however, proceeds in most cases not from the lung-tissue proper, but from the bronchial mucous membrane, and is due to catarrh.

When red-blood cells are present in very considerable quantity, the sputa will be coloured by them. The individual cells are usually intact, and in this they depart from the condition in which they occur in urine and fæces. In some cases, however, as in pneumonia, they are altered, and appear as pale discoidal bodies. Where blood has accumulated for some time in the bronchial tubes, the red cells may disappear entirely, and the pigment remain in the sputum either in irregular particles or as red crystals of hæmatoidin (*vide* fig. 34, *e*). The sputum of pneumonia derives its colour from blood pigment suspended in its substance.

Finally, it is to be noted that in hæmorrhage from the lungs the sputum consists entirely of red corpuscles; in congestion the blood is intimately mixed with mucus.

3. Epithelium.—The sputum abounds in epithelial cells.[3] Squamous cells (fig. 34, *h*) come either from the mouth or from the surface of the true vocal cords. Ciliated epithelium is less often seen, and occurs chiefly in severe bronchial inflammation, when, too, it is probably derived rather from admixture with nasal mucus[4] than from the surface of the trachea, which, as is well known, is lined with ciliated epithelium. The cells as found in the sputum are usually deprived of their cilia (fig. 34, *c*), unless in quite recent expectoration, when cilia in active motion may still be seen upon them. The mere presence of such cells in the sputum is of little diagnostic import; but where they occur in great numbers they may be taken as indicating the commencement of acute catarrh, either in the back part of the nasal fossæ or in the trachea and bronchi.

But there is another variety of epithelium,[5] whose appearance in the sputum is a fact of great importance. It is known as "alveolar" epi-

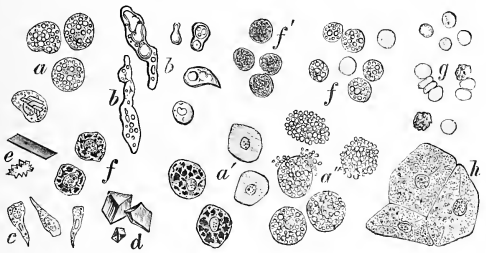


FIG. 34.—Sputum.

a, *a'*, *a''*. Alveolar epithelium.
b. Myelin forms.
c. Ciliated epithelium.
d. Crystals of calcium carbonate.
e. Hæmatoidin crystals and masses.
f, *f'*, *f*. White blood-corpuscles.
g. Red blood-corpuscles.
h. Squamous epithelium.

thelium, a name which will serve provisionally, although its derivation from the alveoli of the lungs has quite recently been questioned (*Bizzozero*).[6] It consists of elliptical cells, each containing one nucleus, which usually requires acetic acid to make it visible. The body of the cell consists of finely granular protoplasm, and very often holds irregular pigment particles in its substance. These particles consist usually of blood colouring-matter, iron dust or carbon (fig. 34, *a'*). In the last case they are unaffected by reagents generally. Iron dust may be known by turning blackish-green with sulphide of ammonium, or blue with yellow prussiate of potash and hydrochloric acid.

These cells often contain one or more fatty granules, readily recognisable by their high refractive power, and at times they exhibit extreme fatty degeneration (fig. 34, *a*, *a''*), when their protoplasm is replaced by

oily drops of varying size. Sometimes large bodies like drops of fat, presumably derived from the rupture of these cells, are to be seen in the sputum (fig. 34, *b*). They were first observed by *Virchow*,[7] who named them **myelin droplets**, from the resemblance they bear to similar forms produced in the destruction of nerve tissue.

According to *Panizza*,[8] however, myelin (which is only the outward form of a considerable number of different substances) is probably mucin; and in his opinion no diagnostic importance is to be attached either to myelin or to myelin-containing cells.

Buhl[9] thought that the appearance of alveolar epithelium in the sputum was characteristic of the disease which he has named desquamative pneumonia. It is certainly a fact that such cells are to be found in great profusion only in quite fresh specimens of caseous infiltration of the lung, whether due to bacilli or not. But then they occur also in pneumonia, in chronic bronchitis, and in chronic pulmonary tuberculosis (*Guttmann* and *Smidt*);[10] sometimes, too, in very large numbers. It follows from their manifestation in processes differing so entirely, that their diagnostic significance is on the whole slight.

[*Troup*[11] believes that the presence of alveolar epithelium in the sputum belongs especially to obstinate catarrhs of the apex, when it will be found associated with columnar and ciliated cells; he observes that since such catarrhs tend in most cases to run into phthisis, we have in this a valuable and early sign of impending danger.]

To examine the sputum for epithelium, a small quantity should be treated with acetic acid, when the characteristic nucleus and nucleolus will become evident; or a specimen may be stained with a watery solution of methylene blue.

4. Elastic Fibres.—These fibres occur in the sputum singly or in bundles, and they are commonly arranged in an alveolar manner (fig. 35). They are of varying length and breadth, dark-bordered, slightly curved, and generally exhibit a double contour.* Their diagnostic value is great, as a sign of serious mischief, pointing to destruction of lung tissue. They occur, accordingly, in tuberculosis, bronchiectasis, pulmonary abscess, and occasionally in pneumonia, while the other symptoms of abscess are wanting. The author has repeatedly found elastic fibres in cases of pneumonia which otherwise ran a normal course, and he supposes that in such cases there was destruction of the pulmonary parenchyma only over a very limited area. It is a notable fact that these fibres are rarely to be met with in the expectoration of pulmonary gangrene, and the reason probably is that they are destroyed

* The figure is taken from a case of pulmonary abscess, which was for several months under treatment in Professor Nothnagel's clinic.

in situ by the ferments formed in that process. The fact was first noticed by *Traube*.

Elastic tissue may be introduced with the food, and so find its way into the sputum. A necessary precaution, therefore, is to direct the patient to wash the mouth carefully after food, and to separate the sputa discharged at meal-times from those to be examined for lung tissue. Even when every care has been taken, however, we are exposed to fallacies from this source, as fibres derived from the food may lie for days in the mouth before they are expectorated. *It is only when the bundles of elastic fibre display the alveolar arrangement that we can be certain of their origin in the pulmonary alveoli, and it is then only that they possess any sure diagnostic significance.*

For their detection, when they occur in considerable quantity, it will suffice to add some potash solution to a little of the sputum placed on a

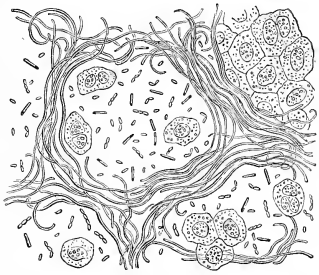


Fig. 35.—Elastic Fibres from the Sputum.

slide. A still better plan is that proposed by *Fenwick*, who boils the sputum in a solution (8–10 per cent.) of KHO, allows the mixture to stand for twenty-four hours in a conical glass, and examines the sediment for elastic fibres under the microscope. [Prolonged boiling with caustic potash will cause the elastic fibre first to swell up and then dissolve. Consequently, the boiling must not be too long. And Dr. Troup (Sputum, p. 48) recommends that when the sputum is placed on the slide, the cover-glass should remain undisturbed.]

5. Spirals.—Certain spiral bodies were first observed by *Leyden*[12] in the sputum of patients suffering from asthmatic paroxysms.

They were afterwards described by *Curschmann* as pathognomonic of disease of the finest bronchial tubes;[13] and since then they have been seen by *O. Vierordt*,[14] *v. Jaksch*,[15] and *Pel*,[16] in the expectoration of pneumonia. More recently *Lewy*[17] [and *Troup*[18]] have written on their occurrence in asthmatic paroxysms.

They are usually discernible by the naked eye when the sputum is carefully examined, appearing as thick white bodies of a twisted and tubular form, and distinguished by their clear tint and great tenacity from all other sputum constituents (fig. 36).

Under the microscope they display a remarkable variety of shape. In the usual arrangement there is a central thread disposed in a more or less zigzag manner, and around it a thick meshwork of very delicate fibres, commonly looped round in spirals, but occasionally retiform. The spirals are often overlaid with epithelium, and sometimes also covered with *Charcot-Leyden* crystals. They vary greatly both in length and breadth. When present, they indicate—so it would seem—a desquamative catarrh of the bronchi (*Curschmann*) and alveoli (*Lewy*). Thus they are found in pneumonia as well as in capillary bronchitis.

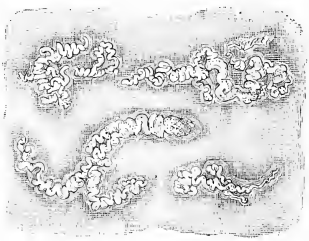


FIG. 36.—Spirals from the Sputum (natural size).

In connection with asthma they afford a most valuable diagnostic sign, showing that the disease is of a bronchial character.

As to the connection between these spirals and the *Charcot-Leyden* crystals (see p. 21 and Chapters VI. and IX.), it is to be noted that in the earlier attacks of bronchial asthma, or at the beginning of a fresh attack, spirals are to be found in the sputum, but no crystals. If, however, a specimen of such sputum is placed quite fresh on a slide, covered to prevent evaporation, and allowed to stand for twenty-four to forty-eight hours, crystals will be seen in it. Later in the asthmatic paroxysm, numbers of similar crystals occur in the fresh sputa amongst the spirals, and not elsewhere. From this it would appear that the *Charcot-Leyden* crystals are in part derived directly from the spirals. As to the chemical constitution of the latter, the author has ascertained that they are formed of a substance closely resembling mucin. If one of them be removed from the sputum and treated with dilute alkali, it dissolves; and when acetic acid is added, a precipitate falls. If the alkaline solution be treated with cupric sulphate and boiled, the result-

ing peroxide of copper is not reduced; but with the previous addition of a mineral acid and boiling, reduction immediately takes place. All these reactions are common to mucin.

Observations which the author had recently an opportunity of making in a case of bronchial asthma went to confirm the statements

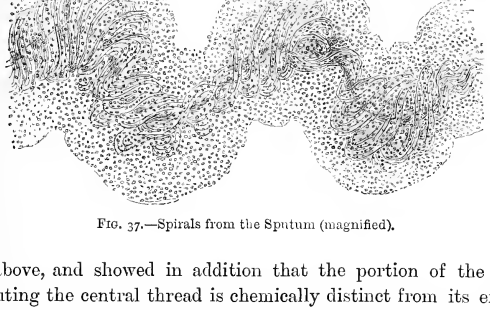


Fig. 37.—Spirals from the Sputum (magnified).

made above, and showed in addition that the portion of the spirals constituting the central thread is chemically distinct from its envelope of mucin. This central thread approached rather to the character of fibrin, but its precise composition was not ascertained with certainty.

6. Fibrinous Coagula.—Fibrinous casts are found in the sputa of plastic bronchitis as well as in pneumonia. They are whitish in

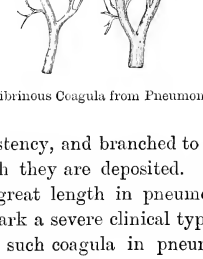


Fig. 38.—Fibrinous Coagula from Pneumonic Sputum.

colour, of varying consistency, and branched to the form of the terminal bronchial tubes in which they are deposited. Ordinarily they are few in number and of no great length in pneumonia (fig. 38), and when more numerous, they mark a severe clinical type of the affection. The abundant formation of such coagula in pneumonia is attended with much coughing and great dyspnœa.

They are found in greatest perfection in the chronic plastic bron-

chitis of adults. They may be said to be pathognomonic of this affection, which is otherwise so often difficult to diagnose. The author has seen them several centimetres long (fig. 39), and minutely branched. Microscopically, their terminal filaments are seen to form a fine network, in which epithelial cells and blood corpuscles are enclosed.

These coagula being formed of fibrin, resist the action of acetic acid. (For their chemical examination, see Chapter VII.)

7. Connective Tissue.—Shreds of connective tissue are occasionally, though rarely, found in the sputum. It sometimes happens in pulmonary abscess and gangrene that scraps of tissue are coughed up,

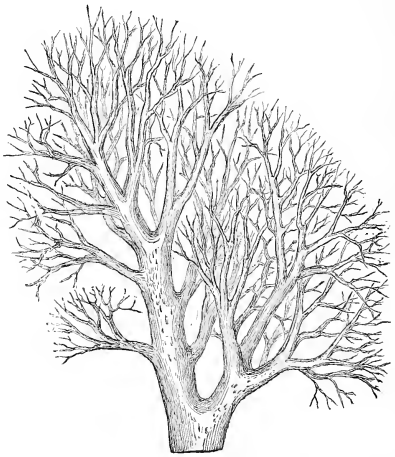


Fig. 39.—Fibrinous Coagulum (Chronic Plastic Bronchitis).

which may be recognised by their arrangement as belonging to the alveoli of the lungs. Fragments of cartilage due to ulceration of the larynx may also be discharged with the expectoration. Their origin can usually be determined by the microscope.

8. Corpora Amylacea.—*Friedreich* [19] has described these starch-like formations as occurring in the sputum. He refers their origin to hæmorrhagic processes in the lungs. They are partly round, partly angular in shape, and contain within them a core of pigmentary substance, which also is usually angular. In a solution of iodine and iodide of potassium, they sometimes give the amyloid reaction, but not always. Often their form shows stratification. In a sputum sent to him for examination by his colleague, *Dr. Neusser*, the author found

such bodies, and he has several times met with others like them, only wanting the dark central substance, in the sputum of pulmonary gangrene. These, however, did not give the amyloid reaction, but they showed evident stratification.

It must still be regarded as an open question whether these bodies are of an amyloid nature or not.

9. Parasites.

1. Fungi.—The investigation of the sputum for fungi has, in recent times, engaged much of the attention of scientists and physicians. If we adhere to the usual classification of these parasites into moulds, yeasts, and fission-fungi, it will be found that micro-organisms of

FIG. 40.

FIG. 41.

Mould Fungi from the Sputum.

the third class have the greatest significance. Of the fission-fungi, some indeed are innocuous; but there are others of a distinctly pathogenic character, and their recognition in the sputum is a point of prime importance in diagnosis. It will serve a useful purpose, therefore, if we divide the micro-organisms in question into two chief classes—the "non-pathogenic" and the "pathogenic."

(*a.*) *Non-Pathogenic Fungi.*

1. Moulds.—There is little to be said of these parasites as a constituent of the sputum. Oïdium albicans (the thrush fungus) rarely occurs (see p. 58), and it is usually to be referred to admixture

with the saliva. In all cases where it is found, the mouth and throat should be carefully examined for aphthæ. At the same time, it must not be forgotten that in thrush aphthous patches may extend to the bronchi, especially in children.

Further, in certain pulmonary diseases mould fungi have been found in the expectoration. The accompanying figures (40 and 41) show such bodies as seen in the fresh sputa of a patient suffering from traumatic abscess of the lung. *Virchow*[20] has published observations bearing upon this subject; and *Lichtheim*[21] found Aspergillus fumigatus in human sputa. This latter organism has been shown by *Schütz*[22] to be connected with disease in the lower animals.

Most writers have concurred in the opinion that mould fungi are only accidentally present in the pulmonary secretion, but quite recently the labours of *Schütz*, based upon *Lichtheim's* experiments upon animals, have gone far to show the possibility that the multiplication of mould fungi may alone cause destructive processes in the lungs. The observations of *Paltauf* and *Lindt*[23] tend to the same conclusion.

To study the nature of these fungi, they should first be found in the sputa by means of the microscope, and then cultivated on bread and nutrient gelatine. Their relation to disease may also be ascertained by experiments upon animals (see Chapter X.). Whether innocuous or not, it is quite certain that mould fungi are frequently present in the expectoration.

2. Yeasts.—Little is practically known of yeast fungi as constituents of the sputum. The author has discovered scattered yeast cells in the pus from a phthisical cavity.

3. Fission-Fungi.

1. Sarcina Pulmonis.—Sarcinæ have been found in the expectoration in various diseases. They were first described in this connection by *Virchow*[24] and *Friedreich*.[25] They are usually smaller than the S. ventriculi.[26] Their presence is of little pathological import (*Fischer*,[27] *Hauser*[28]), but it would seem that they belong especially to extensive ulceration of the lung.

2. Leptothrix.—*Leyden* and *Jaffé*[29] have repeatedly found leptothrices in the sputum (*vide* p. 59). They may be recognised in the so-called fungoid bronchial plugs of fœtid bronchitis by their property of staining in iodo-potassic-iodide solution. These fungoid plugs have been analysed by *Dittrich* and *Traube* (also by *Leyden* and *Jaffé*), who found in them, besides leptothrices, crystals of hæmatoidin, white and red blood-corpuscles, and in many cases quantities of fat-holding epithelium cells and a mass of fatty detritus.

3. Bacilli and Micrococci.—Various forms of bacilli and micrococci

occur in every sputum. A number of these, and amongst them bacilli with terminal spores, are shown in fig. 35.

(*b.*) *Pathogenic Fungi.*

1. Tubercle Bacillus.—The labours of *Robert Koch*[30] have shown that the sputum of tubercle contains definite organisms, which behave in a remarkable manner towards certain staining-fluids; and this author was further of opinion that the organisms in question were the vehicles of the tubercular virus. His conclusions have been most fully borne out by subsequent observations.[31] Nothing more need be said just now to show the great importance of his discovery. We shall return to the subject later, when we come to speak of tubercular sputum (*vide* p. 86).

The tubercle bacillus cannot be seen in the unstained sputum. When a preparation is made in the manner to be described presently, the bacilli appear either separately or, more usually, in groups, as small rod-like bodies, slightly curved, very delicate, and of variable length (1.5μ–3.5μ). They are quite motionless, and spore-formation can often be seen in them. The spores do not stain by the same process as the parent bacilli, and thus it happens that the tubercle bacillus will seem to be broken up into several (2–6) clear oval compartments. This appearance has led some observers (*Lutz*, *Aman*[32]) to suppose that they had to do with micrococci, but by appropriate methods and careful examination the clear outline of the bacillus can be made visible in such cases through its entire length (fig. 42).

Detection of Tubercle Bacillus.—Since the tubercle bacillus was discovered some years ago, very many methods have been suggested for its recognition, as those of *Koch*, *Gibbes*, *Baumgarten*, *Neelsen*, *Balmer*, *Fräntzel*, and *Kühne*. They all alike depend upon the remarkable property which the bacillus possesses of *staining with aniline dyes in alkaline solution, and (unlike the other organisms, pathogenic and non-pathogenic, which occur in the sputum) retaining the dye in after-treatment with acid and alcohol.*[33]

The bacillus may be shown in any of the above ways when the requisite practice has been obtained, but the methods of *Koch* and *Ehrlich* are the best for a beginner to employ.

A. *Preparation of the Staining Fluid.*—It is important that the staining fluid should be freshly prepared for use in every case, both because the aniline dyes are apt to undergo chemical changes when kept for a time in solution, and also because fungi may develop in the fluid itself, and so mislead the observer. It may be made thus:—In a test-tube carefully washed (with distilled water and alcohol) and dried, an alcoholic solution of gentian- or methyl-violet is made * by

* To simplify the method, a concentrated alcoholic staining solution might be kept in readiness.

dissolving the solid substance in 4 or 5 cc. absolute alcohol, and to such a point of concentration that an object cannot be distinguished through the fluid in the test-tube.

In another test-tube, which also must be carefully cleaned, measure out 6 cc. distilled water; add 10–15 drops of aniline oil; mix the two carefully by shaking well, and pass the mixture through a moist filter. To the clear filtrate add a few drops of the alcoholic solution of gentian (or methyl) violet, prepared as above, till a slight turbidity appears. This should clear again after some minutes. Should the mixture not become perfectly clear, however, it will not interfere with the success of the experiment. Thus is obtained the aniline-water, gentian-, or methyl-violet solution (*Weigert-Ehrlich fluid*).

In addition, a watery solution of Bismarck-brown or vesuvin is needed. To prepare this, a small quantity of one of these substances (about as much as would fit on the point of a penknife) is added to a few cc. of distilled water in a test-tube, and dissolved until the fluid is barely transparent. It is then filtered. The filtrate will be employed in the manner to be indicated presently.

B. *Preparation of the Cover-Glasses.*—The cover-glasses to be used must be scrupulously cleaned by washing first in distilled water and then in strong alcohol, and afterwards dried in an exsiccator, or at any rate in a chamber free from dust. A number of such thoroughly cleansed cover-glasses should be kept ready at hand in a glass case. One is now taken up with forceps, previously sterilised by raising to a red heat, and with similar forceps a particle of the sputum to be examined is placed upon the cover-glass—such portions as appear most purulent being chosen—and spread in a layer as far as possible of uniform thickness. Another cover-glass is placed upon the first, and the two are pressed closely together with the forceps, so that the enclosed sputum is spread very thinly. The cover-glasses, still grasped with the forceps, are then separated, and dried first in the air, after which they are passed two or three times through a carbon-free flame from a spirit- or gas-lamp, the preparation surface being kept upwards.

C. *To Stain and Mount the Preparation.*—The cover-glasses, prepared in the manner described above, are now placed in a watch-glass holding the aniline-water gentian-violet staining fluid already mentioned. They should be allowed to float in the solution with the preparation side downwards. When removed, after twenty-four hours, they will be found to be coloured a dark blue. They must now be placed in dilute nitric acid (1 in 4) until the preparation appears to the naked eye no longer blue, but at most a bright green.

The preparation should not be completely decolorised when removed

from the fluid, because there is danger that the bacilli may also be deprived of the stain if they are submitted to too prolonged action of the acid. When removed from the latter, they must be rinsed with absolute alcohol, then dried in the air, and mounted in oil of cloves or Canada balsam.

For microscopical examination Hartnack's objective No. VII. or Reichert VIII. serves best with a little practice. The beginner may use an oil-immersion lens and Abbé's condenser with advantage. If tubercle bacilli are present, they will appear in the preparation as minute rod-like bodies of a blue colour; but if they are few in number, they may be easily overlooked. And it will promote their detection to stain the surrounding substances by immersion in the solution of Bismarck-brown or vesuvin. To do this, the cover-glass, prepared and stained in the manner already described, is placed in the brown staining fluid (made as above), and left in it until the preparation has assumed an evident brownish-yellow tint. It is then washed

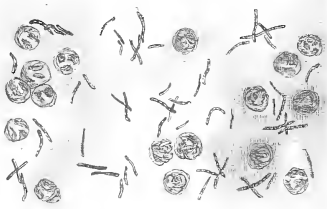


Fig. 42.—Tubercle Bacilli from the Sputum.

with distilled water and mounted for examination as before. When looked at through the microscope, the bacilli are seen to be coloured a dark-blue, whilst the other bodies in the field—fungi, mucus and corpuscles, and epithelium—are brown (fig. 42). The length of the forms found in the sputum is extremely variable. The author has repeatedly met with specimens as large as those shown in fig. 42, but smaller forms, as shown in fig. 43, occur still more frequently. The entire process may be accomplished in a quarter of an hour by heating the staining fluid, which also should then be a concentrated solution of the aniline-water and gentian-violet mixture.

The method of staining with carbol-fuchsin (*Ziehl-Neelsen fluid*[34]) is well worthy of a notice here. Ten cc. of a concentrated alcoholic solution of fuchsin are added to 90 cc. of a 5 per cent. solution of carbolic acid, and the staining-fluid (carbolic solution of fuchsin) is used in the same manner as the *Ehrlich-Weigert* (gentian-violet and aniline) fluid. If the staining-fluid be warmed, the specimen can be

prepared in a few minutes. The surrounding tissues and remaining fungi (non-pathogenic) had best be stained with a watery solution of methylene-blue. The tubercle bacilli then appear red, all other fungi and cells, blue (fig. 43). It may be mentioned here that the modification of this method proposed by *Günther*[35] (in which the fuchsin-coloured preparation is placed in 100 grms. of 25 per cent. sulphuric acid containing 2 grms. of methylene blue) offers no advantages, as the sulphuric acid rapidly decomposes the methylene-blue. We shall have occasion by-and-by to point out the great diagnostic importance which attaches to the discovery of tubercle bacilli in the sputum. [See Appendix X.]

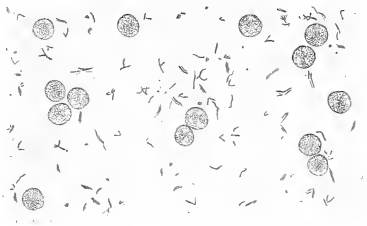


FIG. 43.—Tubercle Bacilli from the Sputum.

2. Pneumonia Microbes.—*Klebs*,[36] *Eberth*,[37] and *Koch*[38] have described various micro-organisms, presumably of specific character, as occurring in the lungs and to be found in the sputum of pneumonic patients.

Friedländer[39] has further investigated this subject, and has succeeded in cultivating and propagating the micro-organisms in question; but their nature is not yet fully understood. They occur in stained preparations in groups of two, three, or four. They vary in size, have usually a distinct envelope, and present the form sometimes of short, stout rods (*Friedländer*[40]), sometimes of diplococci (*A. Fränkel*).

For the detection of pneumono-cocci (see upper part of fig. 44) *Friedländer* recommends a method of staining which closely resembles that of *Günther* for the exhibition of spirilla in the blood (p. 31). The cover-glass preparation, made ready as described above, is passed three times through the flame of a Bunsen's burner, placed for a minute or so in a 1 per cent. solution of acetic acid, the fluid removed by blowing on it through a pointed glass tube, the preparation dried in the air and placed for a few seconds in a saturated aniline-water and gentian-violet

solution; finally washed with water and mounted on a slide for examination. When this is done, a number of rod-like capsulated diplococci may be seen with the microscope.

From a large number of experiments made by *Dr. Richter* at the author's request, it would seem that this method also answers remarkably well for the detection of fungi in exudation or transudation fluids.

Gram's method (*vide* p. 28) will also serve for the detection of pneumonia microbes. These are seen by this method chiefly as small diplococci (fig. 44, round the edges), identical in appearance with the bodies supposed by *A. Fränkel* [41] to be characteristic of pneumonia.

We shall refer later to the pathological significance of these micro-organisms.

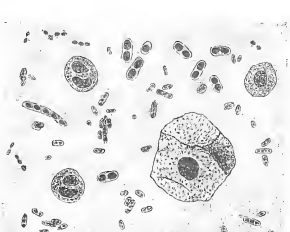


FIG. 44.—Pneumonia Microbes.

3. Actinomyces.—This fungus, hitherto chiefly found in abscesses, appears to settle occasionally in the lungs, and is then apparent in the sputum.

Baumgarten,[42] *Israel*,[43] and *Paltauf* [44] have described such cases. The latter has found the characteristic granular masses in the sputum in cases of actinomycosis, and their peculiar ray-like arrangement is made visible by the application of *Gram's* method. For a description of the ray fungus see the chapter on *Pus*.

It should be mentioned here that organised bodies have repeatedly been observed of late years in the sputum of whooping-cough, some of which may have a causative connection with the disease. Thus *Deichler* [45] has found in such sputa certain amœboid cells (protozoa), but his statement needs further confirmation. *Burger* and *Letzerich* established the presence of bacilli, and *Afanassiew* [46] found in the sputum of affected children bacilli to which he attributed an exciting influence; and since his opinion is supported by *Smtschenko* [47] (who performed careful cultivation experiments to elucidate the subject), it is probable that the bacillus of Afanassiew has really a close relation to whooping-cough.

2. Infusoria.—Infusoria were first found by *Kannenberg*[48] in sputa derived from pulmonary gangrene. They were of the monad and cercomonad varieties. They occurred usually enclosed in small yellow droplets, which also held margarine crystals, and exhibited sluggish movements. Such organisms are described at length in the chapter on *Fæces.*

For their detection in the sputum *Kannenberg*[48] has adopted the following method:—A little of the fungoid substance mentioned above is placed beneath a cover-glass on a slide, and spread out by pressure into a very thin layer. A few drops of a 1 per cent. solution of common salt are then added. The cover-glass with some of the preparation on its surface is next dried, after which it is put to stain in a watery solution of methyl-violet, washed in water, and, while still wet, placed in a concentrated solution of acetate of potash. By this process the protoplasm of the monads is stained a beautiful blue.

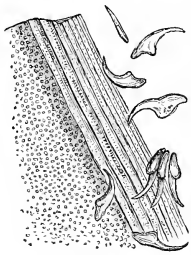


Fig. 45.—Hooklets of Echinococcus and Remains of the Cyst.

3. Vermes.—It very seldom happens that ascarides are found in the sputum, and cysts of echinococcus in a perfect state have been but rarely known to be expectorated. *Eichhorst*[49] and also *Hochsinger*[50] have described cases in which this happened. Diagnosis in such instances cannot be attended with difficulty. The cyst, however, is generally expectorated in fragments; these appear to the naked eye as whitish-yellow shreds, and may be recognised microscopically by their uniformly striped texture (fig. 45). The discovery of the hooklets of echinococcus in the sputum is a fact of great importance in diagnosis. They may be readily recognised (fig. 45) by their characteristic form. Charcot-Leyden crystals in great quantity commonly accompany them.

It would appear, too, that the expectoration may contain the eggs of Distoma hæmatobium. The author is indebted to *Dr. Schiess-Bey* of Alexandria for a specimen which plainly shows that this parasite settles in the lungs, and it follows that when the pulmonary structure

breaks down, it may be expelled with the sputum. Similar observations had already been made by *Manson*.[51]

10. Crystals.—Very many forms of crystals have been found in the sputum, but for the most part their discovery is without great significance in diagnosis.

1. Charcot-Leyden Crystals.—We shall consider these bodies first, for the reason that they seem to possess some pathological interest.

Leyden[52] often found crystals in the sputum of asthmatic patients. They abounded chiefly in the semi-solid greyish-yellow pellets discharged during a paroxysm. These crystals were colourless and of the pointed octohedral form. They were insoluble in cold water, æther, alcohol, and chloroform, but dissolved readily in acetic and mineral acids, alkalies, warm water, and ammonia. They are identical with the crystals to be seen in post-mortem blood, and described at p. 21, and with those of the semen, and with others that are met with in the fæces in cases of anchylostomiasis. According to *Schreiner*[53] such

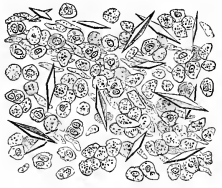


FIG. 46.—Charcot-Leyden Crystals.

crystals are the phosphate of a new base, which, according to the researches of *Ladenburg* and *Abel*,[54] is probably identical with æthyleninim.

Leyden believed that the crystals were the exciting cause of the asthmatic paroxysm. *Friedreich* and *Zenker*[55] found them in expectorated fibrin coagula, and *Bizzozero*[56] in the sputum of acute bronchitis from patients who were not subject to asthma. The author can corroborate the statements of these observers. [*Troup*[57] asserts also that neither spirals nor Charcot-Leyden crystals are pathognomonic of any one disease, but points out that the former are invariably to be found in asthmatic sputa, and by their presence cause the paroxysms. A characteristic feature of the conditions in which both spirals and crystals are found is marked desquamation of the mucous surface of the bronchioles, and the same author refers the origin of Charcot-Leyden crystals to the altered cells which are thus stripped off.]

2. Hæmatoidin Crystals.—Crystals of hæmatoidin, described by *Virchow*, *Friedreich*, and *Schultze*,[58] occur in the sputum as ruby-red

rhombic prisms, either solitary or in groups, or as needles or clusters of needles. These crystals, or fragments of them, are often found enclosed within the substance of white blood-corpuscles (fig. 34, *e*). Under such circumstances hæmatoidin is also seen, either free or in the interior of the white-blood corpuscles, as a mass of pigment in which no trace of the crystalline formation can be discovered.

When hæmatoidin crystals appear in the expectoration, we may conclude that blood has been effused and suffered to remain for some time in the air-passages, or that an abscess has perforated the lung. They occur most abundantly, therefore, in phthisis after hæmoptysis, when pulmonary clots are in process of absorption, very often in pulmonary abscess, and when an abscess or suppurating hydatid cyst has discharged into the lung. *When the crystals are contained in cells, they point to a previous hæmorrhage; but when free hæmatoidin is present in considerable quantity, the inference is that an abscess has discharged from some neighbouring organ into the lung.*

3. **Cholesterin Crystals.**—Crystals of cholesterin were found in the sputum of tuberculosis by *Biermer*,[59] and in pulmonary abscess by *Leyden*.[60] In connection with the former disease they are often to be seen there, but in very small quantity. The author has observed them in great numbers in a girl who had a hydatid abscess in the lung, and in a man suffering from chronic pulmonary inflammation. According to *Black*,[61] cholesterin crystals are plentifully present in the remains of old inflammatory exudation.

These crystals are distinguished by their powerful refractive action on light. They are in the form of large, and often irregular, rhombic tables, which have a tendency to cohere in groups. They are readily soluble in æther; insoluble in water, acids, and alkalies (fig. 106). When the crystals are treated with dilute sulphuric acid and a little tincture of iodine, a violet colour is formed, which presently changes to blue, green, and red. With sulphuric acid alone, they become first yellow and then green, these colours spreading from the edges.

Pathologically, these bodies are of little consequence. They are perhaps most apt to form when pus from some other organ has burrowed into the lung, and lodged there for a time before being discharged through the bronchi.

4. **Fatty Crystals (Margarine Needles).**—These are seen chiefly in putrid bronchitis and pulmonary gangrene. They characterise the discharge of unhealthy pus within the lungs, and belong to bronchiectasis and occasionally to tubercle. They occur either singly or in clusters as long sharp-pointed needles, and are occasionally vaulted or saddle-shaped. They dissolve readily in æther and boiling alcohol, and are insoluble in water and acids. This character facilitates their recog-

nition (fig. 122). Recently the author has found them in plugs, which evidently originated in the crypts of the tonsils (see p. 100).

It follows from their occurring in so many different diseases that the discovery of these crystals in the sputum lends but little aid to diagnosis.

As to their chemical constitution, they consist, most probably, of the sodium, potassium, calcium, and magnesium salts of the higher fatty acids, as palmitic, stearic, &c.

5. Tyrosin Crystals.—*Leyden*[62] first found tyrosin crystals in the sputum of a girl with putrid bronchitis, and again in the case of a man who had an empyema discharging into the lungs. They appear microscopically as fine needles scattered separately or in clusters. As a rule, they occur sparingly in fresh sputum, but form in greater quantity when the specimen has been allowed to stand for some time.

Leyden and *Kannenberg* are of opinion that a profusion of tyrosin crystals implies the existence of a perforating abscess of the lung.

It should be observed, however, that in many instances substances which have been taken for tyrosin were, in reality, lime and magnesia salts of the higher fatty acids.

Tyrosin is usually associated with leucin in the sputum. The latter occurs in faintly lustrous spherical particles (*R. Fischer*).[63] It has the same import as tyrosin.

For the detection of both these substances the method described in connection with the *Urine* may be employed.

6. Oxalate of Lime.—*Fürbringer*[64] has reported a case of diabetes in which the sputum contained large quantities of oxalate of lime. It occurs either in the form of octohedral (envelope) crystals (fig. 92) or as an amorphous conglomerate. *Ungar*[65] found this body in the expectoration of a knife-grinder, aged 28, who had suffered for years from asthma.

The crystals of oxalate of lime are readily known from the fact that they are soluble in mineral acids, and insoluble in water, alkalies, organic acids, alcohol, and æther.

7. Triple Phosphate.—The characteristic coffin-lid crystals have occasionally been met with (fig. 94). They are soluble in acids of all kinds, and are, therefore, only to be found in the alkaline sputum. They are for the most part a product of the decomposition of proteids, attended with the liberation of ammonia. They are commonly to be seen in purulent exudation, and consequently are very plentiful in the expectoration from a discharging abscess. [See Appendix XI.]

Other crystals are also occasionally observed in the sputum. Fig. 34, *d*, represents some that were expectorated in a case of phthisis. They gave the chemical reactions of carbonates (carbonate of lime), the evolution of gas on the addition of acids, &c.

III. CHEMICAL EXAMINATION.—The chemical examination of the sputum throws little light upon disease.

1. Proteids and Allied Substances.—Of these, serum albumin and large quantities of mucin and nuclein (*H. Kossel*[66]) are normally present. Peptone occurs in the expectoration of pneumonia and other purulent conditions—in general, in all cases where the sputa contain pus-cells in abundance.

The best method for the detection of proteids is that recommended by *Hoppe-Seyler*[67] as a test for albumin in serous fluids.

The "prune-juice" expectoration of pulmonary œdema is very rich in serum albumin. For the detection of this body, the sputum may be extracted with very dilute acetic acid, and the filtrate tested with ferrocyanide of potassium, when its presence will be shown by turbidity or a precipitate.

2. Volatile Fatty Acids.—Various volatile fatty acids may occur in the sputum, and notably in gangrene of the lung, acetic, butyric, and sometimes caproic acids are met with.[68] In testing for these bodies, the sputum is diluted with water and phosphoric acid added. The volatile constituents are driven off by distilling them in a vessel heated by steam. The distillate is then tested in the manner laid down in the chapter on *Fæces*.

To separate the fats and non-volatile fatty acids, the sputum is acidulated and extracted with æther, and by repeatedly shaking up the æthereal extract with a watery solution of sodic carbonate, the acids are converted into their corresponding salts, which remain dissolved in the watery solution. The æther is pipetted or siphoned off, and the fats obtained after evaporation of the æther.

3. Glycogen.—This body has been repeatedly detected in the sputum by *Salomon*.[69] Its presence may be demonstrated by *Brücke's* method.

4. Ferment.—*Filehne* and *Stonikow*[70] found that the sputa, especially in pulmonary gangrene and putrid bronchitis, contain a ferment resembling in its action one of the pancreatic ferments. It is soluble in glycerine, and may be extracted by that body from the sputum. *Escherich*[71] has been able to demonstrate its presence in the sputum in all cases of extensive destruction of lung-tissue.

5. Inorganic Constituents.[72]—These are chiefly—

1. Chlorides: of sodium and magnesium.
2. Phosphates: of soda, lime, and magnesia.
3. Sulphates: of soda and lime.
4. Carbonates: of soda, lime, and magnesia.
5. Per-salts of iron (rarely)—phosphate of iron.
6. Silicates.

The inorganic matter of the sputum has little bearing on clinical diagnosis. For its analysis the organic constituents must first be removed by incineration, when the usual tests may be applied to the ash. (For further information consult *Hoppe-Seyler's* Handbuch der Physiologisch- und Pathologisch-chem. Analyse, 5th ed., p. 316.)

IV. THE CHARACTER AND CONSTITUTION OF THE SPUTUM IN THE PRINCIPAL DISEASES OF THE LUNGS AND BRONCHI.

1. Diseases of the Bronchi.

1. Acute Bronchitis.—At the outset the expectoration is viscid, white, and scanty, and occasionally streaked with blood. Microscopically it exhibits very few cells, *and is devoid of specific fungi* (*tubercle bacillus, &c.*). At a later period it becomes more abundant, assumes a pale-green tint, and under the microscope is seen to consist chiefly or entirely of pus-cells. Elastic fibres are never present.

2. Chronic Bronchitis and Bronchiectasis.—The expectoration, which is copious, green in colour, and without any characteristic odour, is formed microscopically almost entirely of pus-cells, with which are mixed a considerable number of fat-holding epithelial cells and myelin particles, and a host of non-pathogenic micro-organisms. When ulceration of the bronchi takes place in the course of chronic bronchitis, leading to bronchiectasis, a very abundant expectoration is discharged in the morning. The fluid, which is usually thin, tends to arrange itself in three layers when allowed to settle. Of these, the topmost is frothy, the next consists of watery fluid, and the third, which is of greater consistence, is formed almost exclusively of cells.

In chronic bronchitis complicated with asthma, the sputum during the paroxysms, and immediately afterwards, exhibits spirals (see p. 69), and *Charcot-Leyden* (pp. 70 and 81) and other crystals.

3. Putrid Bronchitis.—The expectoration of this disease is thick, greenish-brown, and has a very disagreeable sweetish odour. Microscopically it contains a profusion of micro-organisms of various kinds, and very often exhibits large tufts of fungi, which colour blue with iodine and iodide of potassium solution—epithelium usually in the process of fatty degeneration—and fungoid plugs (p. 74), but no elastic fibres, no shreds of alveolar tissue, and no specific fungi.

Lumniczer[73] has separated by means of *Koch's* plate-cultivations (see Chapter X.) a number of micro-organisms from the sputum, amongst them Staphylococcus pyogenes citreus and albus, cereus flavus and albus, and Diplococci; and also a fungus, the colonies of which, when developed in nutrient agar-agar, emitted the odour belonging to the expectoration of putrid bronchitis. It is a spore-forming bacillus,

1.5–2 μ in length, thickest in the middle, and rounded off at the ends. It was also seen ready formed in the sputum. When introduced within the lungs and bronchi of rabbits it excited inflammation there.

4. Plastic Bronchitis.—The sputum contains fragments of the croupous membrane and coagula of fibrin, with which are entangled a profusion of epithelial cells and fungi (fig. 39). From their appearances, and in the absence of pneumonic symptoms, the condition is readily diagnosed.

2. Diseases of the Lung Tissue.

1. Pulmonary Tuberculosis.

(*a.*) **Miliary Tuberculosis of the Lung.**—The sputum in this condition is merely that of acute catarrh. *Tubercle bacilli are never to be found in it.*

(*b.*) **Acute Tubercular Infiltration of the Lung.**—An early diagnosis of the condition is of the utmost consequence, and happily it has been rendered possible by Koch's epoch-making discovery of the tubercle bacillus. Two forms of the disease may be distinguished, according as the symptoms partake of the typhoid or of the pneumonic character.

(*α.*) *The Typhoid Type.*—The symptoms are rigors at the outset, persistent high temperature, enlarged spleen, extensive roseolar rash, which at times suggests that of typhus exanthematicus, and commonly profuse diarrhœa. The physical signs are those of severe catarrh in both apices. There is no dyspnœa. The pulse is frequent, respiration is but slightly accelerated, and lividity is not marked. The sputum is viscid and scanty. It contains but little tissue-débris, and only a few bacilli, which, moreover, are *always provided with spores.*

After the lapse of a few days, dulness on percussion and bronchial breathing may be obtained over the apices. The sputum becomes purulent, and when again examined is found to contain *swarms of tubercle bacilli.* There are also to be seen elastic fibres, showing an alveolar arrangement, and a large quantity of epithelium. The physical signs now give evidence of one or more large cavities, and the fever assumes a remittent character. Death generally ensues in three or four weeks, the mode of dying being that of chronic tuberculosis.

(*β.*) *The Pneumonic Type* begins with sustained high temperature, marked lividity, and accelerated breathing. The physical signs point to catarrh of both apices, and the sputum contains a few bacilli. Later, the characteristic symptoms of infiltration of the lungs set in; the expectoration becomes more profuse, and the contained bacilli more numerous. The disease runs a very rapid course, often lasting only for some days. Anatomically, the condition is one of acute tubercular infiltration of both lungs.

(*c.*) **Chronic Pulmonary Tuberculosis.**—The diagnosis of this disease has been greatly facilitated by Koch's discovery of the bacillus of

tubercle. As a result of his experience of many hundred cases in the clinic of Professor Nothnagel, the author has no hesitation in affirming the principle so often laid down by other authorities, that *the presence of the tubercle bacillus in the sputum invariably implies the existence of tuberculosis.* Let this fact be granted, and no more is needed at once to emphasise the importance of that recent contribution to our knowledge, and to impress upon the physician the necessity of rendering himself familiar with the very simple methods by which the bacillus may be identified (p. 75). Experience has already accumulated certain data. In the first place, it must be mentioned that in most, though not in all cases, the abundance of the bacilli is in proportion to the severity of the disease; and again, the proliferation of bacilli is apt to run parallel to the intensity of the febrile symptoms. The supervention of hæmoptysis is attended with an apparent (*H. v. Frisch*[74]) diminution in their number, but this may well be owing to the fact that the sputa are in such a case diluted by the profuse admixture of blood.

It is only when the tubercular process runs the very rapid course described above that the bacilli, then usually carrying spores, are so abundant as to fill the entire field.

It was held at one time that the appearance of elastic fibres in the sputum was a sure token of incipient tuberculosis, but more recent research has shown that they may be found equally in the course of pulmonary ulceration of whatever kind; and so with other manifestations to which great importance was formerly attached. The significance of all or any of them is very slight in comparison with that belonging to the specific micro-organism, upon the detection of which alone a positive diagnosis can be formed.

It does not necessarily follow from the presence of the bacillus in the sputum that a fatal termination is impending. The author has met with such cases in which the patient eventually recovered, but they are very rare. And doubtless an atmosphere crowded with the bacilli of tubercle (as that of the consumptive ward of a hospital) must necessarily give but little chance of cure. Possibly this is the reason why the hospital physician has so seldom an opportunity of observing it.[75]

2. Chronic (Non-Tubercular) Inflammation of the Lung.—Under this heading may be grouped all those conditions which would formerly have been thought to present the typical character of tuberculosis—fever, night-sweats, &c.—but in which repeated search will fail to detect the bacillus in the sputum. In one such case the autopsy showed caseating masses of considerable extent, but these even to the naked eye were sufficiently discernible from tubercular deposit.

As regards the sputum, its essential characteristic is *the absence of*

tubercle bacilli. It further contains a large quantity of elastic tissue and a profusion of epithelium cells, and especially of their myeloid derivatives. If we may infer from the very slender data which have as yet accumulated, the disease is attended throughout with slight fever, and death by asthenia occurs sooner or later.[76] The author is convinced that, with a little more care, we should learn to recognise the non-bacillary form of phthisis in many instances where at present it is not thought of.

3. **Croupous Pneumonia.**—At the outset of this disease the expectoration is very scanty, of a white colour, and here and there streaked with blood. Microscopically, it exhibits only a small number of red and white blood corpuscles; it usually, though not always, contains the specific micrococci which will presently be described.

Later, and in some cases only a few hours after the initial rigor, the sputum has a rusty tinge. It is usually also at this period remarkably tenacious, and adheres firmly to the spit-box. When placed under the microscope, it is seen to contain but a small number of much-shrivelled blood corpuscles, the red ones arranged for the most part in rows. There are too few to account for the colour of the sputum, which is due rather, as *Traube* surmised, to the presence of dissolved hæmoglobin. In addition to these there are some leucocytes, and the alveolar epithelium which has been noticed in another place (p. 67). In rare cases the spiral bodies already described and shreds of fibrinous coagula may also be observed.

The colour of the sputum at this stage, and even later, may be grass-green, even though there be no jaundice. *Professor Nothnagel*[77] is of opinion that under such circumstances the colouring matter of the blood is changed into bile pigment. At his desire the author has examined some of these grass-green sputa. They were dissolved in a mixture of alcohol with a little chloroform, which was then filtered and the filtrate evaporated, when a substance remained behind which presented all the characters of biliverdin. We may assume, therefore, that the green colour of the sputum in such cases is due to the conversion of hæmoglobin or hæmatin into bilirubin, a change which is the less remarkable if we consider the very close chemical relations which these two bodies bear to one another (p. 40). The bilirubin formed would then be oxydised to biliverdin in the lungs.

Traube maintains that the grass-green sputa belong especially to subacute pneumonia, and that they also occur when a pulmonary abscess forms in the course of pneumonia.

It would appear from the researches of *Rosenbach*[78] in Nothnagel's clinic at Jena, that the sputa may be tinged green by certain micro-organisms, most probably the *Micrococcus chlorinus*,[79] altogether inde-

pendently of pneumonia. This happens under many circumstances; and the fact has in itself no clinical significance.

Later in the course of pneumonia the expectoration becomes more abundant and watery. Its colour changes from brownish-red to a saffron- or citron-yellow, the alteration in most cases depending upon a change in the character of the blood pigment. Fibrinous coagula, and occasionally also spirals, become now more numerous.

It must be noted, however, here, that such saffron- or citron-yellow sputa do not belong exclusively to pneumonia. *Renz*[80] has recorded a case of tuberculosis in which microscopical examination showed a profusion of hæmatoidin crystals in the expectoration, which was of a yellow-ochre tint. *Löwer*[81] also has called attention to a form of sputum essentially distinct from that of pneumonia, though, like it, characterised by a yellow colour, which generally first appears after the sputum has been voided. It occurs, according to *Traube*, almost exclusively during the summer months in connection with tuberculosis, pleurisy, and pleuritic exudation. The colour in this case is due to the presence of micro-organisms, and no special importance attaches to the condition apart from its liability to be taken as a sign of pneumonia.

In the later stages of pneumonia, the fibrinous coagula disappear largely from the sputum, and the red corpuscles and leucocytes are greatly lessened in number and show fatty degeneration. There may still be a few spirals, and elastic fibres are to be met with where ulceration is in progress. Finally, alveolar epithelium, either very fatty, or hyaline (?) [*Feuerstock*[82]], and commonly, too, exhibiting the myelin forms, is present in great abundance.

Lastly, if the disease tend towards recovery, the colour begins to disappear from the sputum, which also under the microscope exhibits a constantly diminishing proportion of epithelium (still, however, fatty), until at last a time arrives when it is indistinguishable from that of an ordinary bronchitis. The subject under consideration would not be complete without some notice of the position which may be accorded to the *pneumonia coccus* of *Friedländer*, when studied as an adjunct to diagnosis. We are not here concerned with the discussion of its effects as an agent of disease, and shall confine ourselves strictly to its significance as a symptom. Upon this point the author has collected the records of a large number of cases which came under *Prof. Nothnagel's* care, and to his researches are added those of *Richter* and *V. Brennerberg*.

It should be stated in the first place that, in every case of pneumonia to which they have been applied, the methods of *Friedländer* and *Gram*, already described, have disclosed the presence in the sputum of bodies resembling the microbe of pneumonia. Since this is true also

of cases of central pneumonia, which are always so difficult to diagnose at the outset, it becomes in the highest degree desirable to assign to these forms their proper significance. It may, perhaps, be assumed that in doubtful cases their presence will confirm the suspicion of pneumonia; but inasmuch as *Friedländer's* microbes (or rather, it should be said, certain forms which closely *resemble* these) have been found in the expectoration of other conditions, such as chronic bronchitis and bronchiectasis, we are never warranted in basing a diagnosis on such manifestations alone. It may well be that the micro-organisms in question are not in all cases identical, and that by improved methods of cultivation we shall be in a position to discriminate them; but at present we are unable to do so. Moreover, it would appear from the researches of *Fränkel*[83] and *Weichselbaum*[84] that there are several distinct microbes, which are capable of giving rise to the symptoms of pneumonia. The researches of *Neumann*[85] have still further extended the limits of the variety which may be observed. It should, however, be mentioned that the researches of *Fränkel*[86] and *Weichselbaum*[87] leave no doubt that amongst the numerous micro-organisms which are present in croupous pneumonia, a diplococcus is most commonly to be found.

Fränkel,[88] *Foà*, *Bordoni-Uffreduzzi*,[89] and *Weichselbaum* have observed the same diplococcus in the purulent exudation of cerebro-spinal meningitis; but it would appear from the researches of *Weichselbaum*[90] and *Goldschmidt*[91] that there are also other forms which appear to be associated in a closer manner with this disease.

To sum up our knowledge, it must be admitted that the subject of the pneumonia microbe needs further elucidation. It is likely that there are several fungi which may excite the disease, and it is only under special circumstances that diagnostic interest attaches to any of them.[92]

4. Pulmonary Abscess.—The sputum of this condition generally looks like pure pus when seen with the microscope. It emits a faint and slightly fœtid odour; and it tends on standing to stratify in two layers, of which the lower consists of pus-cells, whilst the upper is watery and topped with froth. Microscopically the sputum is subject to variety, but in general it exhibits shreds of lung tissue, and very commonly elastic fibres with the alveolar disposition (fig. 35), fatty and disintegrated pus-cells, hæmatoidin—partly in the form of well-shaped crystals, partly as brown or reddish pigment-particles of varying size—often also cholesterin crystals; these are especially abundant in cases where the pus has been of long accumulation. Tyrosin and balls of leucin are occasionally present; fatty crystals are more commonly found; and finally, a profusion of fungi of various forms, but these are never specific.

5. Gangrene of the Lung.—The sputum has a penetrating and extremely unpleasant smell; is abundant, thin, and of a dull-green colour. It separates in standing into three well-marked layers. Of these, the upper is frothy, very turbid, and of a greenish-brown tint; the middle thin, and of a watery or serous character; whilst the lowest is opaque, viscid, and ranges in colour between brown and green. The last occasionally contains brown shreds of lung-tissue of varying size.

Microscopical examination shows that the upper layers contain but few formed elements. In the lowest layer, however, there is a large proportion of detritus, fatty globules of irregular size, rarely crystals, but most commonly hæmatoidin crystals and amorphous masses; an enormous quantity of fungi, notably fission-fungi; often large tufts of fungi, which stain blue with the iodo-potassic-iodide solution (Leptothrix), together with other forms, which resemble starch granules and stain similarly with the same reagent; and sometimes also monads (*Kannenberg*).[93] It is important to notice the absence of elastic fibres. The sputum contains a ferment which acts like pancreatic juice, and it is this which probably causes the solution of elastic tissue (see p. 84).* *Bonome*[94] has constantly found in such cases the *Staphylococcus albus* or *aureus*, which on this account he regards as the cause of this disease.

6. Pulmonary Œdema.—In this condition the sputum is abundant, thin, and watery, and according to the nature of the underlying process is either white and frothy (like soapy water) or of a rusty brown colour (like prune-juice). Microscopically it holds comparatively few cellular elements. The leucocytes and the few epithelial cells to be seen, especially in cases of acute œdema, are free from fatty changes. The red blood-corpuscles are few, and their number is inadequate to account for the deep colour of the expectoration. In one case the author believes that by digestion with water and subsequent filtration of the sputum he obtained the characteristic absorption-bands of methæmoglobin with the spectroscope. The presence of abundance of albumin may be shown by chemical means.

7. Hæmoptysis.—In cases of profuse hæmorrhage from the lung, the sputum consists entirely of bright-red frothy blood, with scarcely any trace of other tissues. When the hæmorrhage has diminished in intensity, the expectoration for several days continues to exhibit a reddish or reddish-brown colour. During this period leucocytes and epithelium are plentifully met with, the cells usually enclosing crystals and amorphous particles of hæmatoidin. The formation of small cavities in the course of tubercle is the commonest cause of pulmonary hæmorrhage.

* [This view is opposed by Troup ("The Sputum," p. 48), who adduces important facts in support of his position.]

In addition to this we shall here notice only the rupture of an aneurism into the bronchi, and remind the reader that a persistent hyperæmia of the lung may also give rise to this condition.

8. **Hæmorrhagic Infarction.**—In cases of recent hæmorrhagic infarction of the lung, single coin-shaped masses of blood, of a bright-red colour, and intimately mixed with froth, are expectorated. After the lapse of several days the sputa assume a brownish tint, and answer the description detailed in the last paragraph (No. 7). Further, as a rule, they contain a number of epithelial cells and leucocytes undergoing fatty degeneration. The cause of this condition is generally to be sought in functional or organic debility of the heart.

9. **Pneumoconiosis.**[95]

(*a.*) **Anthracosis of the Lung.**—The sputum of tobacco-smokers and those who habitually breathe an atmosphere laden with soot always contains some proportion of carbon particles. In such cases the expectoration in the early morning is of a pearl-grey colour, and is brought up in pellets of viscid and remarkably tenacious substance. In a typical case of anthracosis the sputum ranges in colour from dark-brown to black, and is somewhat abundant. Microscopically it exhibits particles of free carbon, readily discernible by their resistance to the action of acids and alkalies; and generally a quantity of leucocytes and alveolar epithelium, in each case choked with pigment particles.

(*b.*) **Siderosis Pulmonum.**—The sputum is usually brownish-black in colour, and has all the characters of that of chronic bronchitis. Under the microscope the contained leucocytes and alveolar epithelium are seen to be laden with a reddish pigment, which by its reaction with sulphide of ammonium (black coloration from formation of sulphide of iron), or with hydrochloric acid and ferrocyanide of potassium (prussian blue) may be known to consist of iron.

(*c.*) **Mason's Lung.**—Here, too, the sputum has generally the appearance of that of chronic bronchitis; but in addition it contains particles of dust, either free or enclosed within cells. Lime and gypsum dust are readily recognised by their chemical properties (see the chapters on *Fæces* and *Urine*) and ultramarine by its colour. In other cases evidence will be forthcoming from independent sources as to the nature of the substance in question.

CHAPTER V.

THE GASTRIC JUICE AND VOMIT.

I. EXAMINATION OF THE GASTRIC JUICE.—Like the saliva, the gastric juice is the product, not of one, but of several distinct sets of glands. It is composed of the fluid secreted by the pyloric glands of the stomach and that of the cardiac glands, these furnishing its active digesting ingredients; and it includes also a proportion of the buccal secretion which has been swallowed, and is already partly changed in the process of digestion.[1]

1. Naked-Eye Characters.—The gastric juice in man is a colourless fluid, generally clear, but occasionally somewhat turbid, and of an acid reaction.

2. Formed Elements.—Microscopical examination of the gastric juice at a time when the stomach contains little or no residue of undigested food, shows some squamous epithelium derived from the upper parts of the alimentary canal; columnar epithelium very rarely, and only in special instances; fungi of various descriptions, especially bacilli and micrococci; and usually also yeast cells. According to *Jaworski*,[2] the microscopical appearances vary as the secretion is acid or otherwise.

If the examination be made while digestion is going on, the character of the gastric juice will approach that of which we shall have occasion to speak later when dealing with the vomit, *i.e.*, it will contain the remains of the food.

3. To Obtain the Gastric Juice.—*Leube*[3] and *Külz*[4] were the first to employ the gastric sound for this purpose in man. Its use is unattended with danger, provided the tubes are elastic, and too energetic aspiration is avoided. To procure the gastric juice for the purpose of chemical analysis, whether in health or disease, the following procedure has been recommended by *E. Schütz*:[5]—

A time is chosen when the stomach is empty, so as to prevent, as far as possible, the admixture of impurities derived from the food; and to this end the morning is generally the best. A pliable gum elastic sound, perforated at the end with a number of minute apertures not larger than a pin's-head, and furnished with a lacquered handle, is

introduced into the stomach, and pushed on until a slight resistance is encountered. A collar of horn is then slipped over its upper part, and grasped between the teeth of the person experimented upon, so as to keep the sound in position. After the lapse of about half a minute the handle is removed and the sound connected with a stomach-pump. The piston is now drawn out, and the projecting extremity of the tube being grasped with the fingers, the sound is withdrawn, and its contents discharged by the movement of the piston into a glass vessel. As already said, the danger of the operation is very slight when performed in this way; but it may be entirely obviated by introducing a mercurial manometer between the sound and the pump, and estimating beforehand the amount of pressure (as indicated by the position of the mercury) which may be employed without applying any considerable suction force to the mucous membrane of the stomach.

It is advisable in many cases, and especially where children are concerned, to adopt the simpler method of *Boas* and *Ewald*,[6] which consists in applying pressure over the abdomen after the introduction of the elastic tube. By this means the fluid is forced into the latter, and may be drawn off as by a syphon.[7] As a substitute for these methods, *Edinger* and *Späth*[8] suggest that the patient should be made to swallow particles of elder-pith stained with appropriate reagents, or leaden balls to which are attached threads stained with the test-reagent.

4. Chemical Constituents of the Gastric Juice.—Of these, the most important are—(1.) Pepsin; (2.) Rennet, or milk-curdling ferment; (3.) Inorganic and organic acids. Each of these is liable to undergo pathological change, both as to quantity and to quality. Those which affect the *pepsin* and *acid* constituents are of chief consequence in disease.

1. Pepsin.

(*a.*) *Detection of Pepsin in the Gastric Juice.*—As a test for pepsin, its property of changing proteids, *e.g.*, fibrin, into peptone is turned to account. The best method of procedure is as follows:—10–20 cc. of the acid fluid, obtained as above, are taken, diluted with water, and filtered. To the clear filtrate a small quantity of well-washed blood fibrin is added, and the whole kept at a temperature of 40° C. If pepsin be present, the fibrin will be dissolved after a few hours. If, after the lapse of 10–12 hours, no change is apparent, or if an odour of putridity be given off by the fluid, it may be assumed that the latter is free from pepsin. Should it happen also that the secretion obtained from the stomach has an alkaline or but feebly acid reaction, it will be necessary, before applying digestion tests, to add to it its own volume of a dilute solution of hydrochloric acid (8 cc. of the fuming acid in 992 cc. of water).

(*b.*) *Quantitative Estimation of Pepsin.*—*Schütz'* method may be employed for the quantitative estimation of pepsin. It is founded upon a principle first enunciated by *Huppert* and *Schütz*,[9] one of fundamental importance for the theory of digestion—namely, that under certain conditions at the disposal of the observer, the quantity of peptone formed is exactly proportional to the square root of the quantity of pepsin used. *Schütz* has taken as the pepsin unit that quantity of the ferment which will yield 1 grm. of peptone under the conditions of his experiment, and expresses his results in terms of this unit. For further details the reader is referred to the original communication.

2. Milk-Curdling Ferment.—This ferment was first investigated by *Hammarsten*. It may be detected by the following process:—2–10 cc. of cow's-milk, of neutral reaction, are well boiled, and to it is added an equal quantity of gastric juice which has been carefully neutralised and filtered. The mixture is placed in a warm chamber, or on a water-bath heated to 30°–40° C. If the milk-curdling ferment be present, the casein of the milk will be precipitated in flakes after the lapse of 20–30 minutes. The ferment was found by *Schumburg*[10] and *Boas*[11] to be invariably present in health, and absent in serious disorders of the stomach, as cancer and atrophy of the mucous lining. It is wanting in infants of from one to two days old; but *Raudnitz*[12] established its presence in older children who were reared on cow's-milk. It was regularly found in a series of investigations conducted on such subjects (*v. Jaksch*).

The researches of *Johnson*,[13] *Boas*,[14] and *Klemperer*[15] have thrown much light on the subject of the rennet constituent of the gastric juice. These tend to the conclusion that the ferment is elaborated by the glands, not as such, but as a zymogen, which then is transformed into the milk-curdling ferment by the action of hydrochloric acid. For the detection of this antecedent substance (zymogen), *Klemperer's* method may be employed:—To 2 cc. of filtered gastric juice are added 10 cc. of milk containing 2 cc. of a 3 per cent. solution of chloride of calcium, and an excess of a 1 per cent. solution of carbonate of soda. The mixture is then placed in an incubator, and if the zymogen be present, coagulation gradually ensues. The amount of the ferment formed varies chiefly with the quantity of hydrochloric acid generated in the process.

Experience has shown that the milk-curdling ferment is in excess in conditions of hyper-secretion and undue acidity of the stomach. When hydrochloric acid is wanting or but scantily present, the ferment is also absent or reduced in quantity; but according to *Klemperer*, the ferment may also result from its zymogen in presence of organic acids.

3. Acids.—The gastric juice contains hydrochloric acid, and also butyric, acetic, and lactic acids.[16]

(*a.*) **Acidity.**—In very rare instances an increased quantity of acid has been found in the stomach,[17] and with this is sometimes coupled an excessive secretion of gastric juice.

Riegel[18] made the important discovery that, *in cases of round ulcer of the stomach, the acid constituent of the gastric juice is greatly in excess;* and the fact has been further established by the observations of *Korczynski, Jaworski,*[19] and many others (see p. 109).

According to *Reichmann, Riegel, Sticker,*[20] and others, a distinction is to be made between hyper-acidity and excessive secretion of the gastric juice. By attending to this point it should be possible better to discriminate between certain affections of the stomach, and especially amongst those conditions which are still classed under the general heading of gastric catarrh, dyspepsia, and the like (see p. 109).[21]

A diminished acidity of the gastric juice occurs temporarily when a large quantity of alkaline substances has been swallowed, and as a persistent condition apparently in all febrile diseases.

The acidity of the gastric juice may be measured in this way:—A certain quantity—increased, if necessary, by the addition of water—is filtered, and its reaction tested. If this be acid, a known quantity of the filtrate is taken and coloured with a little neutral tincture of litmus. Solution of soda of definite strength (the 1/10 normal or deci-normal soda solution may be used with advantage) is now added slowly from a graduated burette, until the point is reached finally at which the onion-red colour of the fluid gives place to a violet hue. From the quantity of soda used, that of the acid present may be known, 1 cc. of the normal soda solution employed in this way corresponding to 0.0365 grms. hydrochloric acid.[22]

The conclusions arrived at by this method are correct only when the gastric juice contains hydrochloric acid alone, and not, as is generally the case, several other acids as well (see below). On this account *Ewald*[23] points out the fallacy of directly deducing the total acidity from the amount of a deci-normal solution of soda required to neutralise it. The expression 50 per cent. acidity implies that 50 cc. of the deci-normal solution of soda will neutralise 100 cc. of the gastric juice experimented upon.

(*b.*) **Hydrochloric Acid.**—The gastric juice secreted during the later stages of digestion appears normally to contain only free hydrochloric acid. At an earlier period lactic acid is also present.

A. Detection of Free Hydrochloric Acid.—The examination of the gastric juice for free hydrochloric acid is attended with much difficulty, since the chlorine salts yield nearly all the same reactions as the free acid. To obviate this, many expedients[24] have been suggested; but we shall notice here only those methods which will serve for clinical purposes.

1. Mohr's Tests.[25]—(*a.*) To the gastric juice to be tested is added first a solution of iodide of potassium and starch-paste, and then a few drops of a very dilute solution of ferric acetate. If free hydrochloric acid be present, a blue coloration (starch iodide) appears. This very simple test is not altogether to be relied upon, inasmuch as it will yield a negative result in presence of phosphoric acid and its salts, even though free hydrochloric acid be present also.

(*b.*) The following test, also discovered by *Mohr*, answers its purpose admirably:—It depends upon the fact that a very dilute solution of ferric acetate, free from alkaline acetates, is unchanged by the addition of a few drops of sulpho-cyanide of potassium solution, and retains its yellow hue, while, if a mineral acid be present, it colours a deep red.

Ewald[26] has obtained good results by applying the test in the following manner:—Two cc. of a 10 per cent. solution of sulpho-cyanide of potassium and 0.5 cc. of a neutral solution of ferric acetate are made up to 10 cc. (with water). A few drops of the solution, which is of a ruby-red colour, are placed in a small porcelain dish, and one or two drops of the fluid to be tested are allowed to trickle slowly on to it. If hydrochloric acid be present, a light violet colour forms at the point of contact of the two fluids, which gives place to a deep mahogany-brown when they mix. This test, according to *Ewald*, has an advantage over the aniline-dye tests in that its result is not materially affected by salts or peptone; but it is certainly less sensitive than the methyl-aniline violet and trapæolin tests.

2. The Aniline-Dye Tests.—(*a.*) **Methyl-Anilin Violet Reaction.**[27]—*Maly* has employed this reagent for physiological, *Van der Velden* for clinical purposes.[28] To obtain the reaction, the fluid to be tested is mixed with a violet-coloured watery solution of methyl-aniline violet. If very much free hydrochloric acid be present (as is never the case in the gastric juice), the fluid will be bleached. If a moderate quantity, it becomes green; and if very little, of a blue colour. The direct examination of the gastric juice never shows more than the transition from violet to blue. For the detection of a very small proportion of acid *Maly* recommends that the mixture should be evaporated to the bulk of one or two drops on the water-bath. So little as $\frac{1}{3}$ mgrm. of hydrochloric acid will then cause the change from violet to blue.

Kost[29] recommends the addition of a 10 per cent. solution of tannin before testing with methyl violet, in order to precipitate peptones, which would otherwise hinder the reaction.

(*b.*) **Trapæolin (00)** in alcoholic or watery solution yields a ruby-red or dark-brown red colour in presence of free acids. *Ewald*[30] considers that this reaction constitutes the most sensitive test for free lactic as

well as hydrochloric acid. *Boas*,[31] who takes the same view, employs a tropæolin test-paper for the purpose.

(*c.*) **Fuchsin.**—The test with fuchsin is far from sensitive, and on that account of little utility.

(*d.*) **Emerald Green.***—The so-called "crystallised" emerald-green affords a sensitive test for free hydrochloric acid. Concentrated solutions of hydrochloric acid give reddish brown, and very dilute solutions a grass or a yellowish green colour with this reagent. Organic acids, as butyric, acetic, and lactic, have no effect upon it, even in concentrated solutions.

A **brilliant-green**, obtained from the same laboratory, has proved a very efficient test. Five mgrms. of this reagent will serve to detect 0.48 mgrm. of hydrochloric acid dissolved in 6 cc. of water, giving to the solution a bright green tint. It is to be noted, however, that a similar effect is obtained where acetic, formic, or lactic acid is present in a higher degree of concentration.

The other emerald-greens produced by *Bayer*, and distinguished as emerald-green (extra crystallised) and emerald green ii. and iii., proved useful, but less sensitive. Of the other reagents tested, Kaiser blue (*Guster*, Berlin) was but little sensitive. Its solutions turned a brown-green with concentrated hydrochloric acid, and an azure-blue with dilute acid. A number of green pigments prepared by *Poirier* of Paris were ineffective as tests for the acid.

Köster[32] has recently employed malachite-green with good results as a test for hydrochloric acid.

(*e.*) **Congo-Red.**—This is an aniline dye, which was first used as a test for free acid by *Herzberg*. It may be employed most conveniently in the form of filter-paper saturated with the reagent, as recommended by *Hösselin*, *Riegel*, and his pupils,[33] for the detection of free hydrochloric acid.[34] This, when immersed in a fluid containing free hydrochloric acid, turns blackish-blue or blue, according as much or little acid is present. This effect is not obtained with organic acids or acid salts in dilute solutions, and the intensity of the reaction is lessened in presence of proteids and of salts in large proportion. The efficiency of the test is undoubted, and notwithstanding that its use is subject to certain fallacies,[35] the Congo-red test-papers must be classed with benzo-purpurin and the aniline-violet reagent as most suited to the purposes of the practitioner.

(*f.*) **Phloro-Glucin and Vanillin.**—The reagent recommended by *Günzburg*[36] contains 2 grms. of phloro-glucin and 1 grm. of vanillin dissolved in 100 parts of alcohol. When hydrochloric acid is added to this, it deposits beautiful red crystals. For the detection of the acid in the gastric juice it is employed thus:—To the fluid to be tested for acid an

* This substance is made at B. Bayer's laboratory, Elberfeld, and, with other reagents, has been made the subject of experiment at the author's request by Dr. Voigt, on whose authority the statement in the text is made.

equal quantity of the reagent is added, and the mixture evaporated on the water-bath. The presence of hydrochloric acid is shown by a delicate rose-red tinge on the surface of the porcelain dish. In this way so little as 0.06 per cent. of the acid is discernible, and the reaction is not impeded by organic acids, albumin, or peptone.

(*g.*) **Benzo-Purpurin** 6 B.—A still more sensitive colour-test is that furnished by benzo-purpurin 6 B. Five mgrms. will serve to show 0.39 mgrm. of acid dissolved in 6 cc. of water (*Hellström*), causing the dark-red colour of the solution to give place to a light violet. A similar change is effected with acetic, formic, and lactic acids; but the colour obtained with organic acids is rather a brownish-violet, and requires a greater quantity of the latter for its production; in the case of acetic acid, not less than 0.84 mgrm. Test-papers may be prepared by soaking strips of filter-paper in a saturated watery solution of benzo-purpurin 6 B, and subsequently allowing them to dry. If one of these be placed in the gastric juice, it will immediately stain a dark blue, provided hydrochloric acid be present in a proportion not less than 0.4 grm. to 100 cc. A brownish-black tint may be due to the presence of organic (lactic or butyric) acids, or from admixture of these with the hydrochloric acid. The ambiguity in this case may be dispelled by placing the paper so stained in a test-tube and shaking it up with sulphuric æther, when so much of the colour as is due to the presence of organic acids will speedily disappear, leaving a lighter stain, or restoring the paper to its original tint. If hydrochloric acid alone be present, no change will be effected in this way, and even after the lapse of twenty-four hours the blue stain will be only slightly displaced. It is important, of course, that the æther used should itself be free from acid. To ascertain this its reaction may be tested with blue litmus paper.

The action of the benzo-purpurin test is not seriously interfered with by peptone and serum albumin, even when these bodies are present in large quantity, and acid salts have no effect upon it.

The following experiments are of interest, as showing the various effects obtained from benzo-purpurin with hydrochloric and organic acids:—

Two solutions were made, one of 4 grms. hydrochloric acid in 100 cc. of water, and another of 6.1 grms. benzo-purpurin 6 B in 600 cc. of water. On mixing together 3 cc. of each, a beautiful blue colour inclining to violet developed, and a coloured flocculent precipitate formed on standing. The addition of hydrochloric acid caused this precipitate to dissolve, and it re-formed on the further addition of the dye.

The same effect was produced whether the solution contained 0.4 or 0.04 grm. hydrochloric acid in 100 cc., 3 cc. being taken in each case. Three cc. of a solution holding 0.004 grm. hydrochloric acid, when added to 3 cc. of a solution of 0.1 grm. benzo-purpurin in 600 cc. of water, gave an evident violet coloration with slight turbidity.

With formic or butyric acid, to obtain the reaction rather less than 0.04 grm.

in 100 cc. of water was required; with acetic acid, something more than 0.04 grm.; with lactic acid, over 0.004 grm. in 100 cc. In all cases alike 3 cc. were taken of each solution.

A comparison of the Congo-red and benzo-purpurin 6 B test-papers shows that the latter are the more sensitive, and they deserve the preference for practical purposes. Hyper-acidity and the preponderance of organic acids in the gastric juice can be shown by this simple procedure in the space of a few minutes.

None of these coloration processes give entirely reliable conclusions. In cases where the reaction is positively obtained, free hydrochloric acid is undoubtedly present; but we may fail to obtain the result when the gastric juice contains albumin, peptone, or salts in considerable quantity, even when free hydrochloric acid is present also. The reactions with methyl-aniline violet, Congo-red, and benzo-purpurin are the most to be depended upon.

3. **Uffelmann's Tests.**—*Uffelmann*[37] has employed the colouring matter of claret in testing for free acids in the gastric contents, and quite recently, as a still more sensitive reagent, the amylic alcohol extract of bilberries, which he applies by means of blotting-paper soaked in it.[38] The reaction depends upon the fact that the colour of such a test-paper changes in presence of hydrochloric acid even when peptone, albuminates, and salts are present, from greyish-blue to a rose tint, which persists after the paper has been washed with æther. (See Appendix XII.)

Lactic, acetic, and butyric acids give similar reactions, but only when in such a degree of concentration as is never found in the gastric juice; and, moreover, the reaction obtained with them is destroyed by the addition of æther.

4. **Ultramarin and Zinc Sulphide.**—These substances were suggested by *Maly*, and employed by *Kahler*[39] as a test for free hydrochloric acid in the contents of the stomach. Ultramarin, according to *Kraus*,[40] is a test for free acids in general. It is decomposed by them even in dilute solutions, sulphuretted hydrogen being given off, while silicic acid and sulphur are precipitated. Zinc sulphide, again, is dissolved in dilute acids with the evolution of sulphuretted hydrogen. It is, however, insoluble in acetic acid.

In testing for hydrochloric acid the process is as follows:—About 20 cc. of the fluid under examination is placed in a crystallising crucible, and so much ultramarin is added as will suffice to give it immediately a blue tinge. The crucible is then covered with a watch-glass, from which depends a strip of filter-paper soaked in solution of sugar of lead, and the mixture is gently heated in the water-bath. After the lapse of a quarter of an hour, if hydrochloric acid be present, the blue colour of the fluid will have given place to a brown tint, while the lead-paper will be stained brown or black. Sulphide of zinc (as much as will fit on the point of a knife) is then added to another specimen, and the same

process repeated, when the brown or black stain upon the lead-paper will again show the presence of hydrochloric acid. The reactions are rendered more feeble by the presence of salts and of phosphates in particular. They can be obtained also with organic acids (lactic and acetic) in more concentrated solutions.

These circumstances and the comparative complexity of the process render its application at the bedside a matter of difficulty. Whereas, on the other hand, we possess in the methyl-aniline violet, benzo-purpurin, and brilliant-green reactions a series of tests which are at once ready and reliable.

B. Quantitative Estimation of Free Hydrochloric Acid.—This can be accurately effected by the very complicated process of *Bidder* and *Schmidt.*[41] All the acids and bases in the gastric juice are quantitatively estimated, the proportion of each in 100 cc. of fluid ascertained, and their equivalents computed. The remaining hydrochloric acid is that which is free in the secretion.

Another method for the determination of this body depends upon the fact that the acid is insoluble in æther, whilst organic acids are soluble in that medium. To utilise this property for the purpose in hand, *Richet*[42] shakes up the gastric juice with æther, and determines by titration the quantity of acid which is taken up by the latter together with what is retained in the watery solution.

Recently, *V. Mering* and *Cahn*[43] have adopted the expedient of collecting the volatile acids by distillation, lactic acid by extraction with æther, and combining the hydrochloric acid separated from the organic acids with cinchonine, shaking up the newly formed hydro chlorate of cinchonine with chloroform, changing the acid into its silver salt, and finally weighing the chloride of silver obtained. *Köster* (see p. 98) has endeavoured to determine the quantity of hydrochloric acid in the gastric juice by a process of titration with alkalies after the addition of methyl-aniline violet.

Günzburg's reagent, according to *Ewald*,[44] will also furnish a means of approximately estimating the quantity of hydrochloric acid present.

Sjöqvist[45] has recently introduced a process for the estimation of free hydrochloric acid in the gastric juice founded upon the following facts: The acids of the secretion may be changed into their barium salts by the action of carbonate of baryta, and when these are incinerated, the baryta salts of the organic acids leave carbonate of baryta, whilst the chloride of barium resulting from the combination with hydrochloric acid remains unchanged. The latter may then be separated from the insoluble carbonate by extracting the ash with warm water, and its quantity estimated by titration with chromate solution. The details of *Sjöqvist's* method are these:—Ten cc. of the gastric juice are filtered and placed in a platinum or silver crucible, and carbonate of baryta *free from chlorides*

added in excess. The fluid is then evaporated to dryness at a gentle heat, and the residue charred and strongly heated for some minutes. After cooling, the residue is treated with 10 cc. of water, the mixture rubbed up, extracted repeatedly with boiling water, and filtered until the filtrate has a bulk of 50 cc. The quantity of chloride of barium in solution is best estimated by titration with bichromate of potash. This body gives with salts of barium a precipitate of barium chromate, which is insoluble in water and acetic acid, and soluble in hydrochloric acid. A solution of bichromate of potash of known strength is added from a burette, until all the barium present is precipitated in the form of chromate. A subsequent excess of bichromate of potash would give to the fluid a deep red colour, which would tend to mask the result. This may be prevented by the use of tetra paper (tetramethylparaphenyl diamine) which has the property of staining blue with oxidising substances. In the process of titration, therefore, the filtrate is mixed with one-fourth or one-third its volume of alcohol and 3–4 cc. of a solution holding 10 per cent. acetic acid and 10 per cent. acetate of soda, and titrated with a solution of bichromate of potash (8.5 to the litre) until a faint trace of blue appears upon the test-paper. The addition of acetic acid and acetate of soda has for its object to promote the precipitation of chromate of barium, and at the same time to prevent the formation of chromate of lime from the small quantity of lime salts and free hydrochloric acid that may be present. From the quantity of bichromate of potash used, that of the barium salt formed, and also of sulphuric acid present, results directly.

It is the author's practice to convert the chloride into barium sulphate, and by weighing the sulphate to calculate the amount of hydrochloric acid in 10 cc. of gastric juice. To that end the gastric juice (10 cc.) is treated with chlorine-free carbonate of barium in excess, and placed on a furnace in a thin porcelain crucible, where it is evaporated to dryness, then gently fused in a muffle; the residue cooled, extracted with boiling water, and filtered; the filtrate evaporated on the water-bath to a volume of 100 cc., and dilute sulphuric acid added. The precipitate (sulphate of barium) is placed on a thick ash-free filter, washed with water, fused in a platinum capsule, thence removed with the usual precautions. The result is calculated thus :—233 parts by weight of barium sulphate ($BaSO_4$) correspond to 73 parts of hydrochloric acid (HCl). And the quantity of the latter contained in 10 cc. of the gastric juice may be calculated from the formula

$$x = \frac{73}{233} \times M = 0.3132 \times M$$

where M = the quantity of barium sulphate obtained from 10 cc. gastric juice

x = the quantity of hydrochloric acid sought in 10 cc.

This method enables the examination to be effected within a comparatively short time.

C. Determination of the Organic Acids of the Gastric Juice.—In this connection we have to deal with lactic, acetic, and butyric acids.

Lactic Acid.—In the detection of this body in the gastric juice the carbolo-chloride of iron test is to be recommended (*Uffelmann*, see *ante*, p. 100), (*Kredel*).[46] To a mixture of 10 cc. of a 4 per cent. solution of carbolic acid with 20 cc. of water, a few drops of perchloride of iron solution are added, and the resulting amethyst-blue colour changes to yellow in presence of a few drops of lactic acid. Alcohol, sugar, and phosphates, however, yield a similar reaction (*Ewald*).[47]

A further and most reliable test for this body is derived from a very dilute solution of perchloride of iron—two to five drops of a watery solution of perchloride in 50 cc. of water.[48] The faint yellow colour of the fluid, whilst not affected by the addition of hydrochloric, butyric, or acetic acid, is intensified in presence of dilute lactic acid. To separate lactic acid from the gastric juice, the distillation residue (see below) of the gastric juice, in which the acid is dissolved, may be extracted with æther, and submitted to the tests described elsewhere (see chapter on *Urine*).

Butyric and Acetic Acids.—If the gastric contents be extracted with æther, butyric and acetic acids may be recognised by their smell (*Uffelmann*). To separate these acids, the gastric juice is distilled and the distillate tested in the manner laid down for the examination of the urine. *Uffelmann* (*ante*, p. 100) directs attention to the importance of a systematic analysis of the gastric juice for the detection of free acids. To do this, the contents of the stomach are filtered and their reaction tested. Should this be acid, they are submitted to the following process:—The total acidity is determined by titration with a decinormal solution of caustic soda, and a portion is tested with dilute solution of perchloride of iron for the presence of lactic acid. Another portion is then tested for free hydrochloric acid with bilberry-dye test-papers. A rose colour obtained when the degree of acidity is slight, and persisting after the addition of æther, indicates the presence of hydrochloric acid. If, on the other hand, the colour is entirely destroyed by treatment with æther, it is evidence of considerable quantities of lactic, butyric, and acetic acids.

Riegel[49] and *Köster* (*ante*, p. 101) have employed similar methods with success; and attention may also be directed to the process described at p. 99.

4. Proteids.—Proteids occur in the gastric contents during digestion, being partly formed in that process, and in part derived from the food. Their recognition affords valuable evidence as to the functional

condition of the stomach; and to make its import clearer, it will not be out of place to refer to certain facts in physiology. The period of digestion may be divided into two stages. (1.) The first of these, which lasts but a short time (15–20 min.), is occupied chiefly with the digestion of starchy matter, and is characterised by the presence of the resulting products, and especially lactic acid. (2.) The second stage commences with the secretion of pepsin and an active gastric juice, by means of which the albumin of the food is changed. The two stages pass gradually into one another, and authorities are not agreed as to whether lactic acid occurs only during the first (*Ewald*, *Boas*, and others) or in the second stage of healthy digestion also, when it is said by some (*Cahn* and *v. Mering*, *Ritter* and *Hirsch*) to be present together with the more abundant hydrochloric acid.

For the purpose of an examination, whether in a healthy individual or otherwise, a test-meal should be administered on an empty stomach. This, according to *Ewald*, should consist of a dry, well-baked roll and water or weak tea; whilst *Leube* and *Riegel* recommend a meal of water-broth,* semolina and flour-gruel,† and meat. Ewald's regimen has the advantage that digestion is at its height within an hour after the food has been taken, whereas in the other case it is necessary to wait for four to six hours before the examination can be begun. The contents of the stomach are obtained in the manner described at p. 93. The proteids in question are albumin, hemialbumose, peptone, and syntonin.

Albumin and *hemialbumose* may be detected by the process detailed in the chapter on *Urine*. Should these bodies and *syntonin* be absent, the biuret reaction (red coloration) will serve directly to show the presence of *peptone*. If, on the other hand, following the method referred to, other proteids (and especially those which are coagulable by heat) are found to be present, these must first be removed in the usual manner (see chapter on *Urine*), provided a sufficiency of material remains to work upon. The filtrate may then be submitted at once to the biuret test, the previous precipitation with phosphotungstic acid not being necessary. (See Appendix XIII.)

Syntonin may be known by its being precipitated by neutralisation from its acid solutions.

About 20 cc. of gastric juice will suffice for an examination of this kind when a little skill has been attained in conducting it. The pathological specimens which come to hand rarely exhibit other nitrogenous bodies than peptone, as is the case also when the contents of

* *Wassersuppe*, translated here as "water-broth," is made of boiling water, with small squares of dry rolls, some salt, and fresh butter.

† *Griessuppe*, semolina soup, consists of semolina boiled in water, and seasoned with salt and butter or extract of meat.

the stomach are examined several hours after the test-meal has been taken.

5. Urea.—For the detection of urea, one of the methods adopted for the same purpose in connection with the blood (p. 46) may be employed. Considerable quantities of this body are found in the stomach in cases of uræmia (p. 51).

6. Ammonia.—Salts of ammonia are abundantly present in the stomach in rare instances. Where a considerable bulk of the gastric contents can be obtained (as by vomiting), the quantity of ammonia present may be estimated, after the removal of the proteids, by *Salkowski's* method.[50] For this purpose, 50 cc. of the vomited matter are taken, 20 grms. of pure powdered chloride of sodium first added, and then 100 cc. of a mixture holding seven parts by volume of a saturated solution of chloride of sodium and one part of acetic acid (1.040 sp. gr.). The whole is then mixed together, allowed to stand for fifteen to twenty minutes, when it is measured and filtered. Of the proteid free filtrate 50 to 100 cc. are measured off, treated with milk of lime, and placed under a bell-glass containing a known quantity of 1/100 normal solution of an acid. After the lapse of from three to five days, the latter is removed and titrated with 1/100 normal alkali solution coloured with rosolic acid. In this way the quantity of ammonia absorbed may be determined.[51]

The same process is applicable to the determination of ammonia salts in the blood and other fluids.

7. Carbohydrates.—Grape-sugar may sometimes be found in the stomach, having either been introduced with the food, or formed there by the action upon starch of saliva which has been swallowed. This latter mode of origin belongs especially to conditions of hypersecretion (*Riegel*, see p. 96, *Ewald*).[52] The mode of testing for sugar is the same here as in the blood (p. 47), the proteids being first removed.

The phenomena of the digestion of starch, and the formation of its intermediate products, involve some points of interest. An hour after food has been taken, under ordinary circumstances, neither starch (blue colour with iodine and iodide of potassium solution) nor erythrodextrin (red with the same reagent) can be discovered in the filtered gastric juice. Should it happen otherwise, some cause tending to delay the amylolytic process may be inferred, and this may be sought either in the fact that the saliva is deficient in diastase, or that there is an excessive secretion of free acid by the stomach at the outset of digestion (*Ewald*, *Boas*, *Rosenheim*).[53] In health, also, when amylaceous food has been taken in quantity, starchy particles may be found, and their nature determined chemically.

5. Estimation of the Rate of Absorption of the Gastric Contents.—The rapidity with which the gastric contents are absorbed, and consequently the functional activity of the stomach in this respect, may be determined thus, after *Penzoldt* and *Faber*.[54] A capsule containing 0.1 grm. of iodide of potassium is given to the patient to swallow. The saliva is then tested for iodine every two or three minutes, by placing a little of it upon filter-paper saturated with starch-paste, and adding a drop of fuming nitric acid. The presence of iodine is shown by a blue colour, which usually appears in 8–15 minutes. According to *Zweifel*,[55] this period is prolonged—thus indicating a deferred absorption—in various affections of the stomach, as dilatation, cancer, and gastric ulcer.

6. A Summary of the Chemical Examination of the Gastric Contents.—It is seldom that sufficient material can be obtained for the systematic examination suggested here, and it will be necessary to make the investigation by successive evacuations of the stomach, the administration of test-meals, &c., before the different processes can be applied.

1. The reaction is to be tested.

2. A known quantity of the fluid, say 10 cc., is taken for the determination of acidity.

3. Another quantity of 10 cc. is examined to show the presence of pepsin and milk-curdling ferment.

4. The benzo-purpurin, Congo-red, and brilliant-green tests for free hydrochloric acid are applied, and the latter estimated quantitatively by *Sjöqvist's* method.

5. A rough estimate is made of lactic, butyric, and acetic acid in the manner described at p. 103.

6. Examination for proteids, this being confined to serum-albumin and peptone where sufficient material cannot be had.

7. Test for starch and its digestion products.

8. The remainder of the fluid is distilled, and the residue shaken up with æther, to determine accurately the quantity of lactic acid which it contains (p. 103).

II. EXAMINATION OF THE VOMIT.

The vomit includes the secretions of the mouth and nasal passages which have been swallowed, and are, for the most part, already undergoing digestion, the gastric juice and ingested substances, in part altered by the action of the stomach, and partly unchanged. Further, it sometimes contains bile.

The naked-eye and microscopical appearances vary with its constitu-

tion, and chiefly with the abundance and character of the food. Apart from such constituents as are derived from the mucous membrane of the mouth and nasal passages, and which have been already described, the vomit almost invariably presents—(1.) Columnar and squamous epithelium, both usually much altered in form; (2.) isolated white blood-corpuscles, generally so transformed by the action of the gastric juice that little more than the nuclei remain; (3.) isolated red blood-corpuscles, usually seen as colourless rings, and very seldom in a quite perfect state; (4.) the following, derived from the food:—

1. Muscle fibres, readily recognisable by their transverse striation.

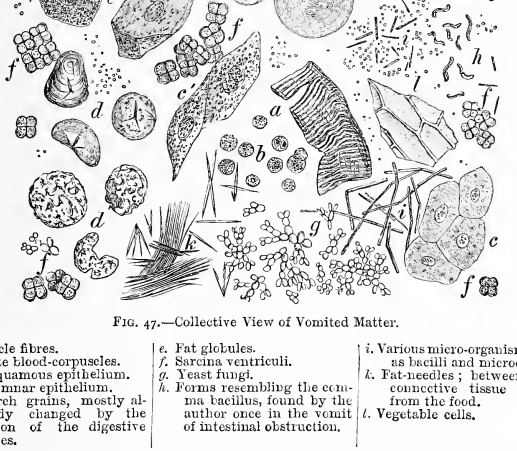


FIG. 47.—Collective View of Vomited Matter.

a. Muscle fibres.
b. White blood-corpuscles.
c, *c'*. Squamous epithelium.
c''. Columnar epithelium.
d. Starch grains, mostly already changed by the action of the digestive juices.
e. Fat globules.
f. Sarcina ventriculi.
g. Yeast fungi.
h. Forms resembling the comma bacillus, found by the author once in the vomit of intestinal obstruction.
i. Various micro-organisms, such as bacilli and micrococci.
k. Fat-needles; between them connective tissue derived from the food.
l. Vegetable cells.

2. Fatty globules and fat-needles, which are sufficiently characterised by their refracting property and their solubility in æther.

3. Elastic fibres and connective tissue.

4. Starch granules, to be recognised by their concentric arrangement and by their property of staining blue with iodo-potassic-iodide solution. These bodies are frequently disintegrated and more or less dissolved by the process of digestion.

5. Vegetable cells of various forms.

In addition, the vomit in disease displays a great variety of fungoid growths (*W. de Bary* [56]), depending upon the nature of the underlying process. Amongst these:—

1. **Mould Fungi** and scattered conidia have occasionally been found. These are, so far as we know, devoid of pathological significance.

2. **Yeasts.**—(*a.*) Saccharomyces cerevisiæ. These are about the size of leucocytes, and refract light powerfully. They cohere in groups of three or more, and stain deeply a brownish-yellow with iodine and iodide of potassium. Very often there are also to be seen elliptical bodies resembling Saccharomyces ellipsoideus (*Rees*).[57]

(*b.*) Very small yeast-like fungi in thick clusters (fig. 47, *g*).

(*c.*) More seldom highly-refracting rod-like bodies, of considerable length and thickness, which generally exhibit a single nucleus. They are rounded off at the ends, and are sometimes separate, sometimes strung together in strands. It would seem that these are the agents in lactic acid fermentation of sugar.

3. **Fission-Fungi.**—The forms belonging to this class are many and various.[58] They include rod-like bodies, which stain blue with iodo-potassic-iodide solution, bacilli and micrococci of every description, and in particular a bacillus which possesses the property of changing glycerine into alcohol by fermentation (fig. 47, *i*).

Sarcinæ ventriculi[59] may easily be known by their shape, resembling that of wool-packs, their dark silver-grey tint, and their property of staining a deep mahogany-brown to reddish-brown with the iodo-potassic-iodide solution (fig. 47, *f*).

After this general view of its microscopical appearances, we shall advert to the physical, chemical, and microscopical characters of the vomit in certain diseases.

1. Acute Gastric Catarrh.—In this condition the vomit consists partly of mucus which has been swallowed, and partly of half-digested food residues. Microscopically it displays the appearances already detailed, which, however, are here subject to much variety, and notably a few red blood-corpuscles are generally to be seen.

Its chemical character varies greatly (*Ewald*).[60] At the outset of the affection, hydrochloric, and commonly lactic, acid in the free state are wanting. The addition of the former will establish a slow digestive process. *Ewald*[61] has been unable to determine the presence of fatty acids in notable quantity, and the proportion of pepsin would appear to be considerably diminished.

The vomit usually is coloured green from the admixture of bile pigment (biliverdin). It often also contains biliary acids. The first may be recognised by Gmelin's test (see chapter on *Urine*), and the latter by Pettenkofer's (see p. 50), or by means of the furfurol and sulphuric acid reaction. (See *Urine*.)

2. Chronic Gastritis and Dilatation of the Stomach.—The vomit is a thin mucous fluid (vomitus matutinus), of alkaline, or it

may be weakly acid, reaction. *Van der Velden* has shown that it always contains pepsin and hydrochloric acid, also organic acids, especially acetic and butyric acids. It is commonly rich in proteids, and notably peptones, which may be easily distinguished by the tests which will be subsequently described in connection with the examination of the urine. Bile pigment is also generally present.

Recent observations seem to show that it is possible to distinguish the different forms of gastric catarrh by the results of the chemical methods described above. Following the conclusions of *Ewald*, we may discriminate (1.) Simple gastritis (2.) Mucous catarrh (3.) Atrophy.

1. In the first form, *simple gastritis*, the test-breakfast is never followed by increased acidity; the proportion of hydrochloric acid is diminished; the secretion contains little pepsin and milk-curdling ferment, and generally, though not always, includes lactic and fatty acids. On the addition of acid the secretion shows digestive activity.

2. In the second form, *mucous catarrh*, acidity is always slight and hydrochloric acid absent; there is abundance of propeptone, but no peptone. Milk-curdling ferment absent. Artificial digestion requires the addition of hydrochloric acid, or the ferment may develop only after a prolonged interval.

3. In *atrophic gastritis* the fasting stomach is usually empty, and its contents, after the administration of the test-meal, is free from mucus, and altogether wanting in pepsin and the milk-curdling ferment.

3. Chronic Ulcer of the Stomach.—The microscopical appearances of the vomit in this disease are those detailed under (2.) in the last section. Otherwise it exhibits nothing distinctive. There can be no doubt that we have in the hyper-acidity which *Riegel's*[62] observations have connected with this condition in a large number of instances, a fact of the highest clinical significance. It may be estimated accurately by the method detailed at p. 101, or by titration. The proportion of hydrochloric acid in the stomach in a case of chronic gastric ulcer is, according to *Riegel*, 0.4–0.6 per cent., as against 0.1–0.2 per cent. in health.[63] It must be mentioned, however, that the researches of *Ewald* [63a], *Ritter* and *Hirsch* [63b], and *Jaworski*,[64] go to show that the increased acidity in connection with round ulcer of the stomach may undergo diminution with the further progress of the disease.

The presence of *blood*, and its character, are facts of great significance.

1. When the hæmorrhage is considerable, clots of blood are found, which are not at all, or but slightly changed.

2. More commonly the effused blood remains for a longer time in contact with the gastric juice, and is thereby altered in such a way that

the oxyhæmoglobin is converted into hæmatin, and the vomit has the appearance of coffee-grounds.

When examined under the microscope in such a case, no blood corpuscles whatever are to be seen in it, but in their place larger or smaller pigment masses. The blood may best be identified as such by *Teichmann's* hæmin test and by the spectroscopic appearances of hæmatin. To obtain the latter, a portion of the vomit should be treated with caustic potash, filtered, and then examined with the spectroscope for the spectrum of hæmatin in alkaline solution (fig. 24).

It should be borne in mind that the exhibition of preparations of iron will impart to the vomit the same appearance as that due to blood; so also will the abundant partaking of red wine; and finally, the presence of bile pigment may cause it to assume a brownish-black colour.

Large quantities of blood (blood pigments) may be found in the vomit in cases of duodenal ulcer with hæmorrhage into the intestine.

4. Carcinoma of the Stomach.—The physical and microscopical character of the vomit in cases of cancer of the stomach are, in general, those of gastric ulcer. Sarcinæ in large quantities are a remarkably frequent manifestation. The blood is very seldom discharged unaltered, and it is usually represented only by colouring matter.

The chemical constitution of the gastric juice in cases of cancer has been made the subject of research by many observers, *van der Velden* (p. 97), *Uffelmann*, (p. 100), *Ewald*,[65] and *Kredel*;[66] also by *v. Mering* and *Cahn* (p. 101); and more especially by *Riegel* (p. 98), *Korczynski*, and *Jaworski*.[67] Absence or diminution in quantity of hydrochloric acid is a point of special interest, and has been recently studied by many investigators.[68]

The author has analysed the contents of the stomach in a great number of cases of cancer, and he has found that in many no trace of free hydrochloric acid could be shown by the colour-tests employed for the purpose. Its absence, however, is by no means so constant as to warrant an absolute diagnosis on this ground alone. Moreover, in other conditions, such as amyloid degeneration of the gastric mucous membrane,[69] in stagnation of the contents of the stomach, and in the febrile state, even without demonstrable disease of the stomach,[70] the hydrochloric reactions may also fail. Taken, however, in conjunction with the other clinical symptoms of cancer, we have in this circumstance important evidence of the disease. *Riegel* points out the important fact that the gastric juice in this affection has entirely lost the digestive property. Concerning the secretion of *pepsin* in carcinoma, it would appear that this, as well as the milk-curdling ferment, are secreted to the end. The tests for pepsin are described at p. 94, and its quantitative estimation may be effected by *E. Schütz's* method.

5. Parasitic Affections of the Stomach.—(*a.*) In but one instance as yet—a case of favus—have the characteristic appearances of this condition been detected in the stomach (*Kundrat*).[71]

(*b.*) Extensive patches of thrush are sometimes formed in the stomach, and in such cases the vomit contains masses of the thrush fungus (p. 58).

6. Croup and Diphtheria.—It very rarely happens that a croupous or diphtheritic condition of the mucous membrane extends from the upper part of the alimentary canal as far as the stomach. When it is so, the vomit exhibits the appearances described at p. 61.

7. Fæcal Substances in the Vomit.—Formed masses of fæces are never discharged by the mouth, but in cases of occlusion or partial paralysis of the intestine, its contents may become mingled with those of the stomach, and brought up with the vomit, which then has an intensely fæculent odour, a yellowish-green colour, and a feebly acid or alkaline reaction. Microscopically it shows nothing distinctive; but on one occasion the author found in such a discharge a quantity of large fungi which closely resembled the comma bacillus (fig. 47).

8. Pus.—In rare cases pus occurs in the vomit. It indicates suppuration in the walls of the stomach, or the rupture into it of an abscess from some neighbouring viscus.

9. Animal Parasites.—Amongst the Entozoa, Ascaris lumbricoides, Oxyuris vermicularis, and Anchylostoma duodenale have been obtained from the stomach. Other worms, such as Trichina, are exceptional manifestations, and the hooklets of Echinococcus and hydatid cysts are occasionally present. *Gerhardt*[72] has found dipteral larvæ in the secretion, where they give rise to the symptoms of gastritis.

10. Constitution of the Vomit in Poisoning.[73]

1. Poisoning with Acids.—In all cases of poisoning with strong mineral or organic acids, the vomit acquires a powerfully acid reaction. It displays a blackened mass of altered blood and charred tissues in cases where the quantity of the poison has been sufficient to cause these effects. The appearance presented in all cases of poisoning with strong acids is the same. To distinguish one from the other in the contents of the stomach, the sense of smell will serve in some instances, as, *e.g.*, for acetic acid, while in others resort must be had to the methods of analytical chemistry. It is important in certain cases to remember that the vomit may contain an abnormal proportion of mineral (hydrochloric) acid, altogether independently of poisoning.

(*a.*) **Detection of Sulphuric Acid.**—The presence of sulphuric acid may be ascertained thus:—The vomit is mixed with a large bulk of distilled water,[74] and put aside for several hours, during which it is frequently stirred. It is then filtered, and the precipitate repeatedly washed with

water on the filter. The filtrate is then collected, and evaporated on the water-bath until the fluid begins to blacken. It is then allowed to cool, mixed with twice its bulk of alcohol, and, after standing for some hours, again filtered. The filtrate, diluted with water, is once more evaporated on the water-bath until the alcohol is entirely driven off. The fluid remaining may then be tested for sulphuric acid. The addition of chloride of barium solution or of lead nitrate should give a white precipitate, showing the presence of sulphuric acid or its salts.

(*b.*) **Detection of Nitric Acid.**—The vomit, which is generally stained somewhat of a yellow hue from the formation of xanthoproteic acid, is mixed with water, boiled, and filtered. The reaction of the filtrate is tested, and if found to be acid, it is neutralised with caustic potash, and evaporated to a small bulk. When allowed to cool, it should deposit crystals of nitrate of potash, which will give the following reactions:—

1. To a solution of the crystals concentrated sulphuric acid is added, and when the mixture is quite cool, a little sulphate of iron solution is poured upon its surface. At the point of contact of the two fluids, a deep brown zone shows the presence of nitric acid. The test is applicable only *where the brown coloration is not obtained with sulphuric acid alone.*

2. A solution of brucin in sulphuric acid is placed in a test-tube, and a little of the fluid supposed to contain nitric acid is poured on its surface. At the point of contact of the two, a red coloration takes place if nitric acid is present.

The modes of testing for **hydrochloric acid** have already been given (p. 96).

(*c.*) **Oxalic Acid.**—The contents of the stomach are partially evaporated and extracted with alcohol, the alcohol evaporated, and the residue, dissolved in water, is treated with acetic acid and solution of chloride of calcium. A precipitate of oxalate of calcium forms, and the characteristic crystals may be distinguished by the microscope.

2. Poisoning with Alkalies.—The vomit in such cases is a viscid, glistening, and strongly alkaline fluid. Where a concentrated solution of a caustic alkali has been taken, the ejected tissues are charred and brown, as in the case of acids. The determination chemically of the character of the poison is often attended with great difficulty, and in other instances again it is very easy.

Where *ammonia* has been taken, the vomit, if examined immediately after the poisoning, will emit the characteristic odour of that body, and further evidence of its presence may be obtained by holding over the fluid a glass rod moistened with hydrochloric acid, when fumes of sal-ammoniac will be given off. The detection of caustic potash and of

caustic soda, on the other hand, involves much difficulty on account of the readiness with which they are converted into their carbonates.

The process for the detection of *chlorate of potash* in the vomit demands a special notice. *E. Ludwig's*[75] method is as follows:—The vomit, if not already acid, is rendered slightly so by the addition of acetic acid, heated, and maintained for one minute at the boiling-point, filtered, the filtrate evaporated to a small bulk on the water-bath, and allowed to settle undisturbed. The salt then separates in the form of crystals, which are dried between folds of blotting-paper and tested in the following manner:—

1. They are treated with dilute hydrochloric acid and warmed; the fluid assumes a greenish-yellow colour, and chlorine gas is evolved. The reaction may be obtained at ordinary temperatures by the addition of strong hydrochloric acid.

2. The crystals are dissolved in water, or, where none have been deposited, the liquid is evaporated and a solution of indigo and dilute sulphuric acid is added. If chlorate of potash be present, on the further addition of a watery solution of sulphurous acid or hyposulphite of soda, the fluid changes from blue to yellow, or altogether loses its colour.

3. Poisoning with Metals and Metalloids.—(*a.*) **Poisoning with Salts of Lead.**—After the lapse of a few hours, a quantity of grey or blackish-grey substance is vomited. For the detection in this of compounds of lead, it should be partially evaporated on the water-bath, and organic matter decomposed by treatment with reagents.

For this purpose *E. Ludwig*[76] recommends the process of *Fresenius* and *Babo*:—The vomit is placed in a large porcelain dish, mixed with about its own weight of a 20 per cent. solution of hydrochloric acid, and 3 to 5 grms. of chlorate of potash added, when the vessel is covered and allowed to stand for about twelve hours. The mixture is then heated to 60° in the water-bath. When the evolution of gas has ceased, more chlorate of potash is added to the brown mass, and this process continued until the fluid ceases to form a brown colour. Should the fluid become unduly concentrated, it should be further diluted with water. If in this way the organic material is not entirely decomposed, hydrochloric acid must be again added, and the requisite quantity of chlorate of potash supplied as before. The mixture is then evaporated on the water-bath until the odour of chlorine is no longer perceived, when it is diluted with twice its bulk of water, passed through a filter which has been moistened with water, washed with a large quantity of water, and the washings finally collected and added to the filtrate. To the fluid so obtained sulphuretted hydrogen is added to saturation. The resulting dark precipitate is then filtered off, washed with sulphuretted hydrogen water, dried, and dissolved in nitric acid in the following

manner :—It is placed in a porcelain dish, and pure (chlorine free) nitric acid is added in drops until the whole has the consistence of a thin fluid, when it is evaporated to dryness on the water-bath, and the residue dissolved in boiling water and filtered. A white insoluble residue of sulphate of lead may remain. This may be reduced to metallic lead by the addition of soda and combustion on charcoal in the reducing zone of a blow-pipe flame.

The addition of sulphuric acid will cause the formation of a *white* precipitate of sulphate of lead, and chromate of potash will give a *yellow* precipitate if lead be present.

The quantitative estimation may be effected in the same way.

Salts of lead in the vomit may be detected by another very simple process :—A strip of magnesium, free from lead, is placed in the fluid, when metallic lead will be deposited upon it, and can then be dissolved in nitric acid, and subsequently proceeded with as above.

(*b.*) **Poisoning with Salts of Mercury.**—Poisoning with compounds of mercury is very often attended with vomiting. The vomit in such cases differs greatly according to the strength of the poison. When large quantities of corrosive sublimate have been taken, pain is experienced in the region of the stomach, and shreds of tissue stained brown with hæmatin are apt to be discharged in the vomit.

The salts of mercury may be detected in the vomit in the same manner as the compounds of lead. Sulphide of mercury is formed in the process, and from this the metal is obtained thus :—To the precipitate carbonate of soda and cyanide of potassium are added, the mixture dried, placed in a test-tube and heated, when the metal is sublimed in the upper part of the tube.

The presence of mercury in the vomit may be shown directly as follows: —Granulated zinc (*E. Ludwig*[77]) or brass wire (*Fürbringer*[78]) is placed in the substances to be examined, which have been previously acidulated with hydrochloric acid; the mixture heated for an hour in the water-bath, and exhausted first with water and then with alcohol, and finally washed with æther and allowed to dry in the air. The brass wire is then placed in a test-tube and heated, when the metal is deposited on the sides of the tube. If now, while the test-tube and its contents are still hot, a small piece of iodine be introduced, the vapour of iodine given off will change metallic mercury into mercuric iodide with the development of a beautiful red colour (*Schneider*).[79] In a similar manner, mercury obtained by other methods may be converted into mercuric iodide. If, at the same time, the vomited matter contains an abundance of organic substances, it will be necessary to remove the latter by *Fresenius* and *Babo's* method before introducing the granulated zinc or brass-foil.

It should be mentioned that in the distillation of the suspected vomit, metallic mercury may pass over with the watery vapour (*Lecco*[80]), and it may also form from the reduction of corrosive sublimate in the process of distillation.

(*c.*) **Poisoning with Salts of Copper.**—When sulphate of copper has been taken, it imparts a greenish-blue tint to the vomit. In poisoning with the copper salts of acetic acid (verdigris, the commonest form), the green colour may be present, or there may be nothing distinctive in the appearance of the gastric contents. The recognition of the poison may be effected by the process described under (*a.*) *Poisoning with Lead Salts.* The sulphide of copper so obtained is dissolved in nitric acid, when the presence of the metal will be shown by the blue colour of the solution, which becomes deeper on the addition of ammonia. If the latter reagent causes a precipitate to fall, the fluid is filtered and the filtrate acidulated with hydrochloric acid. A portion of the filtrate is treated with yellow prussiate of potash, when a reddish-brown precipitate forms. In another portion a piece of iron-foil is placed, and after a little while the metallic copper present is deposited on its surface as a red coating.

It must not be forgotten that traces of copper occur in every organ.

(*d.*) **Arsenic Poisoning.**—The administration of large doses of arsenious acid, Fowler's solution, or of certain mineral waters abounding in arsenic (such as those of Roncegno and Levico), is followed after a short interval by the vomiting of a copious fluid deeply stained with bile. Where arsenious acid (white arsenic) is the poison in question, a careful naked-eye and microscopical examination of the vomit will often afford accurate information as to its nature. In such a case, larger or smaller particles of this substance are usually to be seen. When these white particles are removed with forceps, freed from other impurities by repeated cleansing, washed with cold water, and dissolved in a test-tube containing a small quantity of boiling water, and then allowed to cool, crystals of arsenious acid separate and may be recognised microscopically as small octahedral forms. When the crystals are heated with soda on a piece of carbon in the reducing zone of the blow-pipe flame, the characteristic odour of garlic is evolved, and if a specimen be heated with carbon in a test-tube, a metallic deposit forms in the upper cool part of the tube.

A more accurate method is to remove organic substances with chlorate of potash and hydrochloric acid, then to heat the remaining fluid for a long time at 60° with sulphuretted hydrogen. A yellow precipitate of sulphide of arsenic forms. This is dissolved in sulphide of ammonium, filtered, the filtrate evaporated to dryness, allowed to cool, some drops of concentrated nitric acid added, heated with more nitric

acid until the evolution of gas has ceased and reddish-brown fumes are no longer given off. The fluid is then concentrated to a small bulk in the water-bath, diluted with a little water, and treated with sodium carbonate until its reaction is distinctly alkaline. It is then evaporated to dryness on the water-bath; the dried residue is fused with a quantity of carbonate and nitrate of sodium, allowed to cool, several times exhausted with water, and filtered. The filtrate is repeatedly treated with small quantities of dilute sulphuric acid until effervescence has ceased, when more sulphuric acid is added, and the fluid evaporated first on the water-bath and afterwards over a flame until white fumes are given off. The residue is then allowed to cool and dissolved in cold water. Zinc and sulphuric acid, both free from arsenic, are placed in an apparatus for generating hydrogen.[81] The hydrogen set free is purified and dried by being passed through a tube which is fitted to the apparatus, and contains solid caustic potash and granular chloride of calcium. To the first tube is adapted, by an air-tight connection, another tube constricted in two or three places and terminating in a point. When all the air has been driven out, the hydrogen escaping from the point of the terminal tube is ignited, and then the fluid to be tested is poured into the apparatus. The tube is next heated in front of the place where its calibre begins to diminish, and if arseniuretted hydrogen is mixed with the hydrogen gas evolved, metallic arsenic is deposited in the constricted portion.

A further test is as follows:—The flame is extinguished and the gas conducted into a solution of nitrate of silver. A blackish-grey precipitate of metallic silver separates, and the filtrate, on the careful addition of ammonia, will give a further yellow deposit of arsenite of silver.

(*e.*) **Phosphorus Poisoning.**—Poisoning with phosphorus is always attended with persistent and severe vomiting, which may last for whole days. The discharged substances are free from blood, shreds of tissue, and the other signs of a formidable organic lesion. When solid phosphorus in large quantity has been taken, the gastric contents emit the characteristic odour of phosporus and are luminous in the dark. It should be noted, however, that these properties are lost in presence of alcohol, oil of turpentine, and chloroform.

For the detection of phosphorus, the vomit is distilled with sulphuric acid in the dark, and the retort connected with a glass condenser (*Mitscherlich*). The presence of the poison is shown by the appearance of luminous rings where the phosphorus fumes come in contact with the cold water.

Scherer has also devised an admirable method for this purpose. The vomit is enclosed in a flask provided with an air-tight stopper, and two test-papers—one saturated with nitrate of silver and the other with

acetate of lead—are placed in it. If phosphorus be present, the first of these will be blackened, whilst the other remains unchanged.[82]

4. Poisoning with Alkaloids.*—(*a.*) **Morphia Poisoning.**—The earlier stages of poisoning with morphia are generally attended with vomiting, and in all cases where it has been taken by the mouth, the alkaloid can be detected in the ejected contents of the stomach. *Stas-Otto's* method[83] for its separation may be employed. In this the vomit is placed in a flask, and digested with alcohol and tartaric acid on the water-bath, allowed to cool, and filtered; the alcoholic extract heated on the water-bath at a moderate temperature (60°) until the spirit is entirely driven off, when the remaining watery solution is filtered. The new filtrate is evaporated to a syrupy consistence on the water-bath, and the residue extracted with alcohol. In doing this, the latter should be cautiously added, little by little, until a flocculent precipitate forms, and then in greater quantities until no further turbidity occurs. The alcoholic solution is then filtered, and the filtrate evaporated on the water-bath and dissolved in a little water. The still acid watery solution is next shaken up with æther, in order to eliminate other alkaloids and resinous substances, after which it is rendered alkaline with caustic potash, and again shaken up with æther. Any nicotin and atropin present (see below) are dissolved in this way. The residue is treated with sal-ammoniac and repeatedly extracted with warm amylic alcohol, which takes up morphia. The amylic alcohol extract is next collected, filtered, and evaporated to dryness on the water-bath. The residue is dissolved in acidulated water, which is repeatedly added for the purpose. It is then filtered, extracted with amylic alcohol, neutralised with ammonia, and again extracted with warm amylic alcohol, which is driven off by evaporation. Finally, the residue may be tested thus :—

1. To one portion a freshly prepared solution of molybdate of soda and concentrated sulphuric acid (1 cc. sulphuric acid and 5–10 grms. of molybdate of soda—*Fröhde's reagent*) is added. If morphia be present, the fluid turns first violet, and, changing through blue and green, becomes finally a pale red.

2. Another portion is dissolved in water acidulated with hydrochloric acid, evaporated on the water-bath to dryness, and treated with a few drops of a very dilute solution of perchloride of iron, which should be *free from hydrochloric acid.* A blue colour shows the presence of morphia.

An acid-free solution of perchloride of iron may be best prepared by dissolving the sublimated salt in water (*Ludwig*).[84]

* In this connection we shall consider only such alkaloids as are most frequently the subject of investigation by the physician. For more detailed information on the entire subject, the text-books of *F. C. Schneider*, *J. Otto*, *Kobert*, and *E. Ludwig* may be consulted.

(*b.*) **Poisoning with Nicotin.**—Vomiting frequently occurs in this condition, and the poison may be separated from the gastric contents by the *Stas-Otto* method. The alkaloid is extracted with æther from the alkaline solution of the evaporation residue (*vide supra*), and when the æther is driven off at a low temperature (30° C.) on the water-bath, nicotin remains as a brown or yellow mass. If this be dissolved in æther and an æthereal solution of iodine added, an oily substance forms, from which the ruby-red needles (*Roussin's crystals*) slowly separate.

(*c.*) **Poisoning with Atropin.**—In atropin poisoning with the pure alkaloid, whether taken by the mouth or administered subcutaneously, vomiting rarely occurs; but it is a frequent event in poisoning with the berries of the deadly nightshade. Under such circumstances, the condition is usually rendered evident by the discovery of the berries in the ejected substances, together with the clinical symptoms (mydriasis, &c.). If there should be any doubt as to the character of the toxic agent, recourse may be had to the *Stas-Otto* method, when the alkaloid will be taken up by æther from an alkaline solution of the residue, and may be recognised by the following tests. The æther being driven off:—

1. A portion of the residue dissolved in water, to which a trace of acid has been added, is dropped upon the conjunctiva of an animal (cat or rabbit). After the lapse of from 6–20 minutes paralysis of the sphincter fibres of the iris ensues, and the pupils are widely dilated: 0.01 mgrm. of atropin suffices to produce this effect.

2. A specimen of the residue is dissolved in a few drops of fuming nitric acid, and the solution evaporated on the water-bath: a colourless substance remains, and this, when allowed to cool, and subsequently treated with alcoholic solution of caustic potash, turns first violet, and afterwards a cherry-red colour.

(*d.*) **Poisoning with Ptomaines.**[85]—The ingestion of putrid flesh is occasionally attended with severe symptoms of poisoning, and there can be no doubt that in many cases of so-called acute gastritis, where the use of certain articles of diet—such as liver, kidneys, and oysters—has been followed by nausea, vomiting, and profuse diarrhœa and a retarded pulse, the true cause is to be found in the toxic effects of animal alkaloids. To the same category may be referred the conditions classed under the term "Ammoniæmia" (see p. 51), where the poison may be supposed to develop within the system. Further research is needed for the elucidation of this subject, and in questionable cases the vomit should be tested for ptomaines. In doing this, the observer should be guarded in his conclusions, since peptone (which is normally a constituent of the gastric contents) is known to yield toxic substances of an alkaloid nature.

Stas-Otto's method may be employed for the separation of ptomaines

from the vomit. Such of these bodies, however, as have yet been distinguished exhibit a very remarkable variety in respect of their chemical character. Some may be derived by extraction with æther from an acid, others from an alkaline medium, whilst a third class is distinguished by the fact that its members are soluble only in amylic alcohol, chloroform, or benzol. Others again are insoluble in amylic alcohol. It will be seen from this that the application of the *Stas-Otto* process must be supplemented by extracting the derived substances with various media, and even then it will sometimes happen that the effort is attended with failure.

For such cases *Brieger's* method [86] may be employed. This is briefly as follows :—If the material to be examined contains solid substances, these are first reduced to small particles; sufficient hydrochloric acid is then added to render the whole feebly acid, and the mixture boiled for a few minutes and filtered. The filtrate is evaporated, at first over a flame, and subsequently on the water-bath, until it has attained a syrupy consistence. It may be noted, however, that in view of the instability of the bodies sought, it is advisable to evaporate in vacuum, and at the lowest possible temperature (*v. Jaksch*)—a precaution which, as *Brieger* suggests, should also be taken where foul-smelling substances are the subject of manipulation. The thick fluid is mixed with 96 per cent. of alcohol (filtered), and the filtrate treated with warm alcoholic solution of acetate of lead. The lead precipitate which forms is now filtered off, and the filtrate concentrated—here again preferably in vacuum—to the same consistence as before, when it is again taken up in 96 per cent. of alcohol. The alcohol is driven off by evaporation, and the residue, dissolved in water, is freed from lead by the addition of sulphuretted hydrogen and filtering. The filtrate is acidulated with a little dilute hydrochloric acid, and concentrated (in vacuum) to the consistence of syrup. It is then diluted with alcohol, and alcoholic solution of mercuric chloride added. The resulting precipitate is boiled in water, and certain ptomaines may separate at this stage in consequence of the different solubilities of the double salts of mercury. The better to secure this, the precipitate may be treated successively with water at various temperatures. Should it be thought that the lead precipitate may have retained some of the ptomaines, it may be suspended in water, the lead converted into its sulphide, and the fluid treated in the manner just described.

The solution obtained as above is filtered, and the filtrate, already freed from alcohol and mercury, is evaporated, the hydrochloric acid all but completely neutralised with carbonate of soda, and the residue again extracted with alcohol, after which it is dissolved in water, neutralised with soda, again acidulated with nitric acid, and precipitated

with phosphomolybdic acid. The double phosphomolybdate is filtered off and decomposed by neutral acetate of lead—an object attained more readily by the application of heat on the water-bath. The lead is then removed by means of sulphuretted hydrogen, and the fluid concentrated and treated with alcohol. Several ptomaines are thus separated as hydrochlorates, and may be obtained in the form of double salts of gold or platinic chloride and of picric acid. From these the hydrochlorates are again derived by precipitation with sulphuretted hydrogen; and in the case of the picric acid compounds, extracting with water, acidulating with hydrochloric acid, and finally removing the picric acid by shaking it up with æther.

The next step is to ascertain if any ptomaines remain in the phosphomolybdic acid filtrate after the precipitation of phosphomolybdic acid.

The above is a mere sketch of the process, which, moreover, needs to be modified in many instances.

For the detection of the basic compounds which occur in the secretions as diamins, the best method is that of *Baumann* and *v. Udransky*, in which these bodies are converted into their benzoic compounds by the action of benzoyl chloride and caustic potash. By this means these observers discovered cadaverin (pentamethylendiamine) in the urine, and established the identity of *Brieger's* putrescin with tetramethylendiamine [87] (see chapter on the *Urine*).

Ptomaines (animal alkaloids) yield the characteristic reactions of alkaloids; but otherwise they are not known to possess any distinctive chemical or physiological properties.[88]

The characteristic reactions which are common to all the alkaloids are the following (*Otto, E. Ludwig*).[89] With—

1. *Iodine and iodide of potassium solution*, a brown flocculent precipitate; most readily obtained from the solution of the alkaloid which has been acidulated with sulphuric acid.

2. *Mercuric and potassium iodide*, a white or yellow precipitate, insoluble in water and dilute acid.

3. *The iodide of bismuth and potash*, an orange precipitate in a solution acidulated with dilute sulphuric acid.

4. *Phosphomolybdic acid*, a bright or brownish yellow precipitate, insoluble in water and dilute mineral acids.

5. *Metatungstic, and phosphotungstic acids*, a white flocculent precipitate, with difficulty soluble in water and dilute acid. (This, according to *E. Ludwig*, affords a particularly sensitive test.)

6. *Tannin*, in neutral or feebly acid solutions, a yellow or white precipitate.

7. *Platinic chloride*, a whitish-yellow or citron precipitate, which is sometimes readily soluble in water, and but slightly so in alcohol.

8. *Chloride of gold*, a yellow or whitish-yellow precipitate, which may be amorphous or crystalline.

The animal alkaloids already distinguished as occurring in the human system are sufficiently numerous. They have been detected in

the fæces, the urine, and the milk, and they will find appropriate notice under the separate headings in this work. They are known by their effects both in health and disease, and, in many instances, have been separated as definite bodies from the secretions. Such products result from the decomposition of articles of food. Thus *Vaughan* [89a] obtained one of these bodies (tyrotoxin) from rotten cheese and bad milk, and he supposes that the substance in question is diazobenzol. *Ehrenberg* [89a] found a similar body in putrid sausages. Special mention should be made also of ptomato-atropin, a basic compound, which has been discovered in the latter food. In all cases where the clinical symptoms are those of poisoning, and include severe vomiting, the methods described above may serve to elucidate the matter; but it should not be forgotten, meanwhile, that the presence of peptone may be a source of ambiguity, since it may yield similar poisons.

5. Poisoning with Ethylic Alcohol.—In acute poisoning with alcohol (ethylic alcohol), the vomit emits the characteristic odour of that substance. For its more accurate recognition, the gastric contents are first diluted with water, and, if very acid, carefully neutralised with caustic potash, and then distilled by steam.

The distillate may then be submitted to the following tests:—

1. To a portion a few drops of benzoyl chloride and a little caustic potash are added. When the mixture is heated and again allowed to cool, the presence of alcohol is shown by the characteristic odour of benzoylethylic ether (*Berthelot*).[90]

2. With a small portion an equal volume of concentrated sulphuric acid is cautiously mixed, a little powdered sodium acetate added, and the mixture heated. The characteristic odour of acetic æther shows the presence of alcohol (*Otto, E. Ludwig*).[91]

6. Poisoning with Chloroform.—Chloroform may be detected either directly in the vomit or in the fluid obtained from its distillation. In either case, the following reactions will disclose its presence:—

1. A little thymol dissolved in caustic potash is added to the fluid to be tested, and the latter is then heated. If chloroform be present, the preparation assumes a dark violet tint (*Vitali* [92]), and if β-naphthol be used instead of thymol, a blue colour results (*Lustgarten*).[93]

2. A few drops of alcoholic solution of caustic potash and a little aniline are heated with the distillate of the gastric contents. If chloroform be present, isocyanphenyl is formed, and may readily be recognised by its unpleasant odour (*Hofmann*).

In a case of poisoning with chloroform, where the drug was taken by the mouth, the author was unable to find any trace of it in the substances vomited three hours afterwards, although the toxic symptoms were well marked.

7. Poisoning with Carbolic Acid.—When a poisonous dose of carbolic acid has been administered by the mouth, the vomit usually emits the characteristic odour of that substance. The presence of carbolic acid in the ejected substances may be ascertained directly by the following tests:—

1. Bromine water yields with a fluid containing carbolic acid a yellow crystalline precipitate of tribromophenol.

2. A solution of perchloride of iron colours dark violet in presence of carbolic acid.

In testing for these reactions, it is well previously to filter the vomit, if necessary washing it upon the filter with water. If the reactions are not obtained in this way, the filtrate is distilled with a little sulphuric acid, and both tests are again applied to the distillate.[94]

It is to be borne in mind that carbolic acid may form in considerable quantity in the intestine in certain morbid states, and may then, as in intestinal obstruction, find its way into the vomit. (See chapter on the *Urine.*)

8. Poisoning with Nitro-Benzol and Aniline.—(*a.*) *Nitro-Benzol.*—The presence of nitro-benzol in the vomit may be detected frequently by the characteristic odour, which resembles that of the oil of bitter almonds. To separate this substance from the gastric contents, the latter is distilled with a little sulphuric acid. The distillate will contain oily drops, which are soluble in æther. Aniline may be derived from nitro-benzol by the addition of granular zinc and dilute hydrochloric acid. When reduction is effected in this way, the fluid is rendered alkaline with caustic potash, and the aniline formed is extracted with æther. The oily residue after the expulsion of the æther will yield the following reactions:—

1. If the aniline solution be treated with hydrochloric acid, and a shaving of pine-wood be placed in it, the latter assumes a deep yellow colour.

2. A drop of the oil is suspended in water, and a few drops of dilute solution of chloride of lime or of a very dilute solution of sulphide of ammonium added: the fluid assumes a rosy-red colour (*Jacquemin*).[95]

3. A very sensitive test is that of *E. Ludwig*:[96]—A watery solution of aniline colours a dark blue on the addition of a watery solution of carbolic acid and hypochlorite of soda; and this colour changes to red on the further addition of hydrochloric acid.

4. The isocyanphenyl test serves well for the detection of aniline formed in the decomposition of nitro-benzol. It was introduced by *A. Flückiger* as a test for acetanilide (antifebrin). To the fluid under examination a few drops of caustic potash and chloroform are added. It is then shaken up and heated, and when again allowed to cool, should emit the disagreeable odour of isocyanphenyl.

(*b.*) *Aniline.*—Poisoning with aniline also is often attended with vomiting. The vomit is diluted with water, and distilled with a little sulphuric acid. The distillate is extracted with æther, and when the latter has been driven off by evaporation an oily substance remains, to which the above tests (1 to 4) for nitro-benzol may be applied.

9. Poisoning with Prussic Acid.—This condition may usually be recognised by the characteristic odour of oil of bitter almonds. For the detection of the poison the vomit is treated with a small quantity of tartaric acid, and distilled. Hydrocyanic acid passes over with the distillate. From its presence, however, poisoning with the drug is to be inferred only when it can be ascertained that the vomit is free from innocuous double salts of cyanogen, as, for instance, the yellow or red ferrocyanide of potassium.

The best test is to add solution of ferric chloride and sulphate of iron to a little of the filtered fluid. With this last reagent yellow ferrocyanide of potassium gives a white precipitate, which soon changes to a bright blue, while with the perchloride of iron it forms a Prussian-blue precipitate. Red ferrocyanide of potassium gives a dark blue precipitate with sulphate of iron, and a deep brown coloration with the perchloride. Should both the above-mentioned substances be present, the following process may be applied:[97]—The fluid is acidulated with sulphuric acid, and treated with an excess of carbonate of lime. The corresponding lime-salts take the place of ferro- or ferri-cyanide of potassium, and only so much hydrocyanic acid as is not combined in the double salts of cyanogen passes over with the distillate. The latter is then tested for hydrocyanic acid in the following manner:—

1. To a few cc. rendered alkaline with caustic potash, a few drops of a freshly-prepared solution of cupric sulphate are added; the mixture is heated, maintained for the space of one minute at boiling-point (*Ludwig*), and allowed to cool. It is then rendered strongly acid with hydrochloric acid. A blue coloration of the fluid results, and when allowed to stand for some time, a flocculent Prussian-blue precipitate settles in it.

2. To a few drops of the distillate a yellow (holding poly-sulphide of ammonium) solution of sulphide of ammonium is added, and the mixture boiled until the yellow colour entirely disappears. It is then allowed to cool and perchloride of iron and hydrochloric acid added. If hydrocyanic acid be present, the preparation assumes a red colour (sulphocyanide of iron). *E. Ludwig*[98] applies this test in a different manner:—The fluid is treated with yellow solution of sulphide of ammonium in excess, a few drops of caustic potash added, and the mixture evaporated to dryness. The residue is exhausted with water, treated with hydrochloric acid, and filtered. The filtrate is then tested

with solution of perchloride of iron, when a blood-red colour should develop.

3. Another very admirable test has lately been suggested by *Vortmann*.[99] To the fluid to be tested a few drops of nitrite of potash are added, and then two to four drops of the solution of perchloride of iron, and finally dilute sulphuric acid, until the yellowish-brown colour of the basic ferric salt formed in the beginning of the reaction has changed into a light yellow. The solution is then heated to boiling, allowed to cool, treated with ammonia and filtered, and to the filtrate a small quantity of a colourless solution of sulphide of ammonium is added. The presence of a minute quantity of hydrocyanic acid will be shown by a bluish-green, of large quantities by a beautiful violet-red colour. *Vortmann* has named this the "nitro-prusside test."

The vomit produced by a number of other poisons, as carbonic oxide gas, sulphuretted hydrogen, &c., exhibits no distinctive properties.

CHAPTER VI.

THE FÆCES.

UNDER the term fæces[1] are comprised all those substances which, being formed from the food in the process of digestion, and mixed with the residues of the secretions of the alimentary canal, are finally expelled from the body by the rectum.

I. NAKED-EYE CHARACTERS OF THE FÆCES.—The character of the fæces varies considerably in health, depending chiefly as it does on the nature of the food ingested. The labours of *Nothnagel*, however, have obtained for us a knowledge of some points which are more or less characteristic of healthy stools. Such a stool is moulded, and of a certain consistency. Its reaction is sometimes alkaline, sometimes acid. It is alkaline in certain morbid states, as, *e.g.*, typhoid; but in others, as the acute enteritis of children (and, in the author's experience, of adults as well), the reaction is acid. The so-called "clayey" stools of dyspepsia in children, however, are nearly always strongly alkaline,—a fact which is due to the presence in them of carbonate of ammonia. Nothnagel concludes that there is little to be learned from the reaction of the fæces.

The colour, too, is very inconstant. It depends upon the food taken, and is greatly modified by drugs supplied to the system. When, *e.g.*, bilberries are eaten freely, the fæces are coloured black. So, too, they are rendered black by preparations of iron, manganese, or bismuth, the colour in these cases being due to the formation of the sulphides of those metals. After a meal of cocoa-nibs or chocolate, the stools are apt to be coloured grey (*Widerhofer*).[2] The exhibition of calomel will turn them green, an appearance which was formerly attributed to the formation of sulphide of mercury, but which is now known to be caused by the presence of biliverdin (*Betz, A. Vogel, Monti, Zawadski*).[3] *Lesage*[4] distinguishes two varieties of the green stool of children. The colour in the first is due to biliverdin, but in the second it is caused by a definite bacillus which produces a green colour-substance. This bacillus can be cultivated outside the body, and inocu-

lated upon animals. Its presence in great abundance in the fæces is associated with a severe form of cholera infantum. Santonin, rhubarb, and senna will stain the fæces yellow.

The presence of unaltered bile pigment in the fæces is *always* pathological (*Pettenkofer*).[5] Healthy excrement contains a pigment which has been named stercobilin (*Vanlair, Masius*[6]), and which *Maly* asserts to be hydrobilirubin (urobilin). This latter body can be prepared artificially from bile pigment, and it is probable, *à priori*, that a similar change is effected in the process of intestinal digestion, when we would expect to find urobilin in the fæces. For a further description of this body and the tests by which it may be recognised, see p. 39, and the chapter on *Urine*.

The quantity of fæces passed by a healthy man in twenty-four hours averages 120–200 grms. The remains of undigested food are often to be found in the excrement, as berries, fragments of potatoes and apples, and shreds of fibrous tissue. *Virchow*[7] relates a case in which orange-skins voided with the fæces were taken for a parasite, and *Eichhorst*[8] one in which great coils of hard, woody asparagus were passed unaltered by digestion.

Amongst the occasional constituents of the fæces visible to the naked eye, must be mentioned the cylindrical shreds of mucous membrane, of larger or smaller size, which are passed in the affection known as tubular intestinal catarrh (enteritis mucosa or membranacea), (*Nothnagel*).[9] The cases in which such mucous formations are found are not very rare. Their discharge is attended with violent tenesmus, and unaccompanied by the passage of fæces. They are ribbon-like or reticular in form, and consist chemically of mucin and fibrin (*Litten*).[10] They are often of great length. In one instance they had the appearance of coiled brownish-yellow strings, 0.5 c. thick, with here and there shreds of transparent membrane. The material under description was readily compressible beneath the cover-glass, and when looked at through the microscope, exhibited a profusion of transformed intestinal epithelium involved amongst long, spirally-wound, and twisted threads. These bore some resemblance to the *Curschmann-Leyden* spirals (p. 69), but had no central thread, nor did they exhibit crystals. In all probability their presence implies chronic catarrh of the large intestine, usually associated with obstipation.

A patient, suffering apparently from chololithiasis, whilst under the author's observation passed with the fæces a shred of tissue 5 cm. long and 3 cm. broad, which proved to be intestinal mucous membrane. No cause could be assigned for its detachment.

Virchow and *Nothnagel*[11] describe the occurrence of bodies resembling frog-spawn or cooked sago-grains in the stools, and some observers

have supposed that they were derived from ulcerating intestinal follicles. *Virchow* thinks they come from an excess of farinaceous food. *Nothnagel* has also met with particles about the size of a poppy-seed, which chemically had the constitution of mucus. It must, however, be mentioned that this author has never found mucin in any quantity as a constituent of healthy excrement.

Foreign bodies of all kinds are occasionally to be seen in the fæces of lunatics and children.

Finally, tumours or parts of tumours originating in the alimentary canal, and stones or concretions formed in the gall-bladder or intestine, may be passed with the stools.

The presence of gall-stones is a fact of great clinical interest. They may in all cases be detected by a careful examination with the naked eye.

The fæces of a woman suffering from various symptoms of dyspepsia were found to contain a number of soft, white and yellowish particles about the size of a pin's-head. These proved to contain cholesterin and carbonate of lime, and it is probable that they were multiple small biliary concretions.

II. MICROSCOPICAL CHARACTERS OF THE FÆCES.—To study the microscopical appearances of the fæces, a small particle should be pressed between a cover-glass and slide. If the stools are fluid, a drop may be placed upon a slide, and so examined. Much, of course, depends upon the character of the food, and the following description applies to the fæces of an adult living chiefly upon animal diet.

1. Constituents Derived from the Food.

(*a.*) **Vegetable Cells.**—These are of very variable form, and occur separately or in organic connection with one another. They sometimes exhibit starch granules or remnants of chlorophyll (fig. 48, *e-i*, *l*).

(*b.*) **Muscle Fibres** are almost invariably found in the fæces. Their quantity depends upon the supply of flesh-meat. They are relatively fewer after a mixed diet (*Nothnagel*).[12] They are usually much altered, stained yellow by the bile, and swollen; but with a high power of the microscope, their transverse striation can usually be made out.

(*c.*) **Elastic Fibres** are readily to be distinguished by their double contour and curved form. They are always derived from the food, and occur both in health and disease.

(*d.*) **Areolar Tissue** is found occasionally in the excrement of persons who eat much animal food, and in this connection is of no pathological consequence. But if areolar fibres occur plentifully where flesh is sparingly eaten, they afford an indication of digestive disturbance.

(*e.*) **Fat.**—Fatty globules are occasionally seen, but fat occurs more

commonly in needles, arranged separately or in clusters (*Nothnagel*). Such needles are found in greater quantity when much fat has been taken with the food. The stools in alcoholic poisoning are always rich in fat, so also with the fatty diarrhœa of children.

(*f.*) **Starch Granules.**—These particles may be easily detected by their reaction with a solution of iodine and iodide of potassium. They are commonly to be found, but sparingly and of small size, in healthy stools. They are also found in the interior of vegetable cells. *Nothnagel* asserts that an excess of such substances in the excrement points to a derangement of the intestine.

(*g.*) **Coagulated Proteids.**—Undigested milk occurs in the stools, especially in children and in persons suffering from diarrhœa. *Noth-*

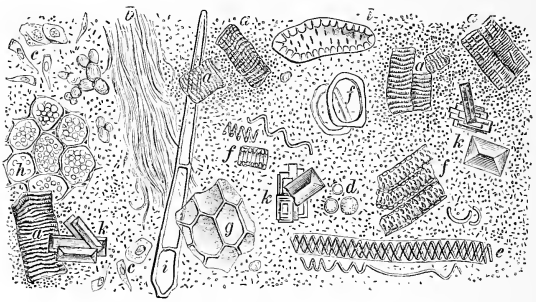


FIG. 48.—Collective View of the Fæces.

a. Muscle fibres; *b.* Connective tissue; *c.* Epithelium; *d.* White blood corpuscles; *e.* Spiral cells; *f*—*i.* Various vegetable cells; *k.* Triple phosphate crystals in a mass of different micro-organisms; *l.* Diatoms.

nagel has described a substance resembling coagulated albumin which is occasionally to be seen in cases of intestinal trouble. It occurs in round yellow particles, varying from the size of a bean to that of a millet-seed. Their substance is readily soluble in a 5 per cent. solution of HCl, is precipitated from alcoholic solution by acetic acid, and redissolves in excess of the acid, from which it may be again precipitated by ferrocyanide of potassium. They resemble the mucous particles of *Nothnagel*, to which reference has already been made. That author thinks that the substance in question is casein.

The discharges of infants at the breast are of a very different character from those of adults. Here muscle fibres, areolar, and elastic tissue are absent, coagulated proteids taking their place. Microscopically they abound in fat and crystals of the fatty acid salts.

2. Formed Elements Derived from the Intestinal Tract.

1. Red Blood-Cells.—Red blood-corpuscles are rarely to be found in the fæces. *Nothnagel* has examined the freshly-voided excrement of typhoid, when it was deeply stained with blood, but could find none of these cells. In such hæmorrhagic stools, however, are seen larger or smaller masses of reddish-brown pigment (hæmatoidin), and the rhombic crystals of hæmatoidin are also occasionally present. In cases where the blood is derived from the upper part of the alimentary canal or has remained for a long time in the intestine, the fæces are no longer red like blood, but are stained a dark brown or black. We have already seen that a similar effect may be produced by certain drugs (see p. 125), and consequently it is not possible to infer a hæmorrhage from this appearance alone; neither will red blood-cells be visible under the microscope. In such cases the presence of blood may be determined with absolute certainty by the application of *Teichmann's* test to a dried particle of the fæces (see p. 39).

FIG. 49.—Degenerated Intestinal Epithelium.

2. Leucocytes.—In healthy stools leucocytes are rarely to be met with. They are always loaded with fatty particles. As a pathological condition, the appearance of leucocytes in great quantity in the excrement is far from common. *Nothnagel* found absolutely no increase in the number of these bodies in simple intestinal catarrh. When a considerable increase does occur, it affords a presumption of ulceration of the gut.

Pure pus is found when an abscess has discharged into the intestine and in dysentery.

3. Epithelium.—Epithelium is always found in the fæces in health. Squamous epithelium comes from the region of the anus. Columnar epithelium is more rarely met with. (Fig. 48, *c*.) The latter is usually uncoloured, but occasionally stained yellow. The cells occur either separately or in masses. Their boundaries, as a rule, are not easily to be recognised, but well-formed goblet-cells are sometimes seen (*Nothnagel*). Sometimes, too, they contain fat, and are then very large.

Altered epithelial cells are often found in the fæces. In their typical manifestation they constitute what Nothnagel has termed "fusiform degeneration." They are, for the most part, small, non-nucleated, homogeneous, and somewhat glossy bodies, tending towards the spindle shape (fig. 48), but exhibiting every variety of form between this and the ordinary epithelial structure. *Nothnagel's* opinion is that these bodies are epithelial cells altered by abstraction of fluid, and he remarks that he has found them most typically shown in the mucous coating of scybalous masses. The mere presence of epithelium in the stools is a fact of no clinical consequence. When in disproportionate quantity, it points to intestinal catarrh.

4. Detritus.—There are certain indeterminate substances always to be found in the fæces, which are usually either derived from the food or belong to the waste products of digestion. They occur separately or in masses, and are little affected by reagents, although sometimes they are soluble in alcohol. Their constitution is very various.

3. Parasites.—There is no part of the body so apt to be infested by parasites as the intestine. Such parasites belong both to the animal and the vegetable kingdom, and it is not unreasonable to conclude, from the vast numbers in which some of them are always to be found, that they exercise an important function in the final processes of digestion. This may be said especially of certain vegetable forms, and among them the fission-fungi, presently to be mentioned, hold a prominent place.[13]

A. **Vegetable Parasites.**—It will be useful to divide these parasites, as before, into two classes,—the pathogenic and the non-pathogenic; but in doing this, it must be premised that some of the organisms classed as non-pathogenic may also at times be closely associated with morbid states. The non-pathogenic parasites will first engage our attention, and we shall adopt the usual classification into moulds, yeasts, and fission-fungi (*bacteria*).

1. Non-Pathogenic.

1. Moulds.—The only specimen of this class which has yet been found in the intestine is the thrush fungus. It occurs in children suffering from thrush, and its presence in the stools is clinically of no significance.

2. Yeasts.—Yeast cells (*Saccharomycetes*) are the commonest form of parasite in the intestinal discharges, whether of health or disease (*Nothnagel*), (fig. 48, between *b* and *c*). *Uffelmann*[14] asserts that yellow yeast-fungi are often also to be seen in the fresh stools of infants at the breast. Micro-organisms of this kind are most abundant in the acid stools of children. They are round or oval; lie together in groups of three or four; commonly exhibit their characteristic sprouting arrange-

ment. Well-formed yeast-fungi, however, such as are seen in fermenting saccharine solutions, are very rarely to be met with. *Nothnagel* saw such once in a case of typhoid in a child. In the bile-stained and acid discharges of acute catarrh of the small intestine in adults the author has not infrequently found fungi which closely resembled the form described by *Rees*[15] as the *Saccharomyces ellipsoideus*, except that they were somewhat smaller (p. 108).

The yeast-fungi of fæces stain a mahogany-brown with the iodo-potassic-iodide solution. This property depends upon the fact that they hold glycogen in their substance.

There are other forms to be met with morphologically resembling yeast cells, but distinguished from them by giving a blue colour with the iodo-potassic-iodide solution mentioned above (see p. 132).

3. Fission-Fungi.—These organisms exist in swarms in the intestine, and they are to be found in greater profusion in the fæces than in any other of the excretions (*Nothnagel*, *Brieger*, *Uffelmann*, *Escherich*, *Bienstock*, *Stahl*, *Kuisl*, *Miller*, *Sucksdorf*[16]); indeed, it is not incorrect to say that they always constitute the bulk of these discharges. Bacilli and micrococci of the most varied kinds are the commonest forms. They occur separately or in colonies, and often exhibit lively movements. It may be said, as a rule, that micrococci predominate in the solid, and bacilli in the fluid motions. The former are sometimes found in shapes resembling torulæ and sarcinæ. The organism which occurs most commonly and in greatest abundance is the *Bacterium termo*, and there can be little doubt that this body is intimately associated with putrefactive processes in the intestine; although it must be stated at the same time that we are ignorant of its functions in health, where also it exists abundantly, and that the host of other micro-organisms which make their appearance when putrefaction is going on throws a great deal of doubt over this question.

Bacillus subtilis is found in the discharges both of health and disease. It was first discovered by *Nothnagel*, and may be seen as long threads with spores attached, or as separate spore-bearing rods, and elsewhere the spores alone occur in clusters. Its relatively thick edges and the remarkably glossy appearance of the spores facilitate its detection. Its presence has no clinical significance.

The various micro-organisms above alluded to stain brown or brownish-yellow in solution of iodine and iodide of potassium or of ammonium iodo-iodide; and this property belongs especially to the groups of micrococci, which are coloured very deeply a brownish-yellow by contact with this reagent.

In addition to such forms, the fæces exhibit other micro-organisms, which stain blue or violet in the iodo-potassic-iodide solution. *Nothnagel*

has described many of these, and one especially which he holds to be identical with the *Clostridium butyricum* of *Prazmovski*.[17]

The author has investigated this subject with great care, and he is in a position to confirm *Nothnagel's* statements in every particular, and would venture to add something to his description of the fungi which stain blue with the iodo-potassic-iodide solution.

To begin with the minutest forms. There is a micrococcus which occurs in swarms of uniformly and finely granular zoogloeform bodies, which colour a reddish-violet with the solution.

Next in order is a micro-organism in the form of short delicate and somewhat pointed rods, which recall the *bacillus of septicæmia in the mouse*, and which stain in a similar manner with the reagent mentioned. These rods occasionally contain one or two spherical granules, which do not stain in the solution.

There are other rods, of varying length, which resemble the *Leptothrix buccalis* in the manner of their reaction with the iodine fluid; and a further variety, which differs from the *Bacillus subtilis* only in that the fungus threads stain blue, while the bodies referred to above as the spores remain uncoloured (fig. 50).

The author has often had an opportunity of observing the *Clostridium butyricum* of *Nothnagel*. It takes the form of large round cells, usually visible in uncoloured preparations by reason of their somewhat lustrous appearance, and in other respects resembling yeast-fungi. In some cases they adhered to one another like a string of beads, and in others were simply disposed in groups (fig. 51).

A micro-organism of oblong and somewhat pointed form also occurs in the fæces.

It must be mentioned that although these bodies all stain some shade of blue with the solution of iodine and iodide of potassium, the tints which they derive from that reagent exhibit notable differences; for whilst, on the one hand, the micrococci are but slightly coloured, and of a purple tint which tends towards red, the rod-like micro-organisms stain very deeply, and of a dark-blue colour. The stain is in all cases transitory, fading in from twenty-four to forty-eight hours, and disappearing altogether within a few days.

Those fungi whose characteristic it is to stain blue in iodo-potassic-iodide solution occur probably in all stools, but in relatively small numbers in those of health. They are in profusion only in certain morbid states, and especially in intestinal catarrh. They do not seem to bear any relation to the reaction of the discharges, having been found alike when this was alkaline and when it was acid. To conclude, they are constantly present in health and in disease, and in the discharges of infants at the breast, as well as of older children nourished on a meat-diet.

Hence it follows that no definite clinical significance attaches to any of the parasitic forms hitherto described. It is a matter of observation that in certain pathological conditions of intestinal derangement one form or the other is apt to preponderate, but we have no evidence that they are in any case the cause of disease, or that the multiplication of a particular micro-organism is not rather the consequence of the disturbance which it is found to accompany.

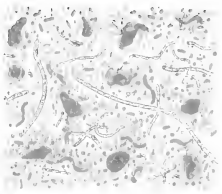


Fig. 50.—Fungi from the Fæces staining Blue with the Iodo-Potassic-Iodide Solution.

The intestine is apt to be infested with microbes of a pathogenic character, which in form closely resemble those innocuous parasites which have been just described. Much light has been thrown upon their nature by the researches of recent years, and for their detection we have to make use of a number of special methods. But these alone will not suffice. Without an accurate knowledge of the commoner, at least, of the non-pathogenic organisms which normally inhabit the

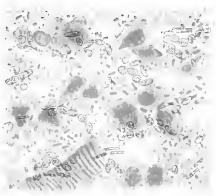


Fig. 51.—Fungi from the Fæces staining Blue with the Iodo-Potassic-Iodide Solution.

intestine, the discrimination of the others is impossible. It is for this reason that we have dwelt at some length—though still in a far from exhaustive manner—on the description of the innocuous micro-organisms which are most frequently to be found in the fæces.

2. Pathogenic Fungi.—We pass now to a consideration of the pathogenic parasites, the bacilli of cholera, typhoid, and tubercle.

1. **Cholera Bacillus** (*Comma Bacillus*).—To *Robert Koch*,[18] the

pioneer of the modern science of bacteriology, belongs the honour of having first discovered the micro-organism which causes that most terrible of the epidemic diseases of our time—cholera.

We shall not attempt to give an account of all that has been written upon this subject, but shall content ourselves with making here and there a reference to the leading authorities for the information of the reader; and we shall avoid the discussion of controverted points of which we have no personal knowledge. *Nevertheless, there can be no doubt whatever of the fact that a definite and morphologically distinctive parasite occurs in the discharges of cholera patients;* and it is a task at once difficult and of the utmost importance to define the form of this organism, and to devise methods for its recognition.

Koch described the cholera bacillus as a short rod-like form, curved or semicircular, and somewhat thicker than the bacillus of tubercle. Such bodies often lie two together, with the concavity of their curves turned in opposite directions and their extremities in contact, so as to

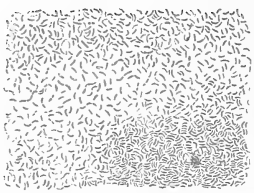


Fig. 52.—Cholera Bacilli (pure cultivation).

form an S-shaped figure. They produce by division peculiar screw-like spirals, which remind one of the spirillum of relapsing fever, only that these are thicker (fig. 52). Koch did not find any separate spores belonging to these organisms, but it would seem that *Hueppé*[19] has succeeded in doing so. Such bacilli were discovered by Koch in the intestine and discharges, rarely in the vomit, of Asiatic cholera, and never in the blood, saliva, tears, urine, or breath of patients suffering from this disease. The discharges, according to this authority, sometimes contain almost unmixed colonies of bacilli. Koch's statements have been borne out by the subsequent investigations of *Babes, Vandyke-Carter, Nicati, Rietsch, van Ermengen*, and others.[20]

It follows from what has been said of the enormous profusion in which micro-organisms of various kinds infest the intestine, that the cholera bacillus, when not very plentiful, may easily escape detection; and therefore it will not suffice to submit discharges which are supposed to contain it to a simple microscopical examination. To meet

the exigencies of the case, Koch has studied the mode of growth of the bacillus, and he has devised for its cultivation certain special processes, on the application of which the diagnosis of Asiatic cholera may very safely rest. The first requisite is to separate some of the bacilli or their germs from the mass which contains them, and this may readily be done in the manner to be described in another part of this volume (*vide* chapter on *Methods of Bacteriological Research*).

A complete and exhaustive search for the cholera bacillus may be performed as follows :—

1. A particle of the discharge is placed upon a slide, and examined microscopically for the bacillus. In doing this, the expedient of *Schottelius*[21] may be adopted with advantage. He puts a little of the stool in an open glass, together with an equal quantity of alkaline meat-broth, and allows the mixture to stand at a temperature of 30°–40° C. for twelve hours. At the expiration of that period, the bacilli will be found in swarms on the surface of the glass, and a specimen may be obtained which will consist almost entirely of them.

2. A particle of the discharges or a drop of the infected broth (*Schottelius*) is next spread out in a very thin layer between two cover-glasses, dried, passed three times through the flame of a Bunsen burner, stained with one of the basic aniline dyes (fuchsin, methylene blue) and examined under the microscope.

3. Plate-cultivations are made from the suspected stools with nutrient gelatine and agar-agar, as described in Chapter X.

4. If comma-bacilli develop in these, cultivations are to be made by inoculation in the depth of nutrient substances.

5. The bacillus is to be cultivated in hanging drops (*vide* Chapter X.)—(it is well to apply this test at once, in the event of comma-bacilli being found in Schottelius' preparation)—and the micro-organisms developed in the medium are to be compared with those derived by plate-cultivation.

When the discharges examined belong really to Asiatic cholera, the processes 1 and 2 will generally show the comma-bacillus of *Koch*. The inference to be drawn from the third process depends upon the fact that the cholera bacillus, cultivated on a nutrient gelatine plate at 22° C., forms after twenty-four hours white colonies, with irregular, jagged, or sinuous outlines. These exhibit a light-yellow or rosy tint, and present an appearance like that of a layer of powdered glass overlying the gelatine plate. The colonies grow gradually darker towards their centre, and presently begin to liquefy.

Plate-cultivations in nutrient agar-agar form a greyish-yellow, furrowed, slimy surface, and do not cause the underlying nutrient medium to liquefy.

When cultivated by inoculation in the depth of the nutrient substance contained in a test-tube (see p. 135), after the lapse of twenty-four hours the nature of the bacillus is shown by a white coloration, which extends along the track of the needle, and the formation of a funnel-shaped cavity, which increases gradually from the circumference, and acquires the appearance of an air-bubble. It is only at its superficial part that this liquefaction occurs, the lower layer of the inoculated substance remaining unaltered for days.

In hanging-drop cultivations, the comma bacillus exhibits the following peculiarities (see p. 135):—When examined on the day following the inoculation, or, it may be, after the lapse of a few hours, and with an oil-immersion lens and a constricted diaphragm, mobile swarms are seen in the centre of the mass, while at its circumference appear the spirochæta-like bodies, which sometimes exhibit as many as twenty spiral twists. Suppose that a specimen, which Schottelius' method had previously shown to contain micro-organisms when cultivated in this manner, displayed some forms which resembled the comma-bacillus, it will then be necessary to transfer some of these drop-cultivations in the manner described in Chapter X. for cultivation by the plate and deep-inoculation methods.

Bujwid has recently recommended his chemical process (see below) in combination with that of *Schottelius* for the purpose of disclosing the cholera microbes, even without the aid of the microscope.

It should be mentioned that the cholera bacillus will thrive at a temperature of 37°, and even on boiled potatoes. Its cultivations resemble those of the bacillus of glanders to the naked eye, but their growth is slower and needs artificial heat. The bacilli are very sensitive to drying and to exposure to a 5 per cent. solution of carbolic acid.

Bitter and *Rietsch* [22] have shown that the cholera microbes elaborate a peptonising ferment.

Poehl and *Bujwid* [23] found that the addition of a 5 to 10 per cent. solution of hydrochloric acid will, in a few minutes, impart to cholera cultivations, and to no others, a violet-rose colour; and *Brieger* [24] has been able to separate from them a colour-substance, to which he gives the name of "cholera red," but which *Salkowski* [25] identifies with indol. *Bujwid's* cholera reaction does not seem to merit the commendations bestowed on it. Other fungi, pathogenic and innocuous, behave in a similar manner with mineral acids.

Cantani [26] notices, as a result of his experiments upon animals, that cholera bacilli elaborate a poison; and *Brieger* [27] has actually separated from its cultivations specific toxic substances, which occur together with cadaverin and putrescin, and result from the agency of cholera

microbes. The method he employed is that detailed at page 119. It remains for the clinical observer to detect these substances ready-formed in the discharges of cholera patients; and some progress has already been made in this direction (*Pouchet*).[28]

There are two other micro-organisms which bear a considerable resemblance to the comma-bacillus. One of these, the bacillus of cholera nostras, is a pathogenic micro-organism; the other is *Deneke's* spirillum of cheese.

2. The Bacillus of Cholera nostras.—*Finkler* and *Prior*[29] have observed a micro-organism resembling the comma-bacillus in the discharges of cholera nostras. It is distinguished from the bacillus of Asiatic cholera chiefly by its size (fig. 53), being both longer and broader than the latter. In addition to this, the two exhibit notable differences in life-history. When a colony of the *Finkler-Prior* bacillus is cultivated on a plate in nutrient gelatine, it is seen to be of a uniformly round figure with well-defined edges; and when examined

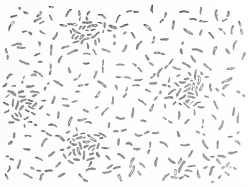


FIG. 53.—Bacilli of Cholera nostras (pure cultivation).

with a low or medium power, it has a granular appearance, and is usually of a brown colour. Moreover, the gelatine rapidly liquefies, and emits a very foul and penetrating odour.

The comma-bacillus of *Koch*, on the other hand, develops less rapidly. The colonies are never coloured brown, but of a tint ranging from light-yellow to rose-colour; and the figure which they form, as already mentioned, is bounded with a ragged contour (see p. 135).

Again, when cultivated by inoculation in the depth of nutrient substances, this bacillus develops in a very characteristic way; for whilst the comma-bacillus swarms in the funnel-shaped manner already described, the cultivation in this case assumes a saccular shape, somewhat comparable to that of a stocking.

The pathological import of the *Finkler-Prior* bacillus is still a matter of dispute, but it is plainly of the utmost consequence to be able to distinguish this comparatively harmless organism from the very fatal microbe of Asiatic cholera.

Spirillum of Cheese.—*Deneke*[30] found a micro-organism in ripe cheese which bears a close resemblance to the comma-bacillus of *Koch*. But this, like the *Finkler-Prior* bacillus, can be distinguished by certain peculiarities in its life-history. The nutrient gelatine medium is rendered fluid sooner than by *Koch's* bacillus, but more slowly than by that of *Finkler* and *Prior*. This micro-organism, moreover, will not develop in potato, while the other two will thrive upon this food. The crucial test, however, is the result of inoculation upon animals. *Deneke's* bacillus has no morbid influence on the intestine.

3. Bacillus of Typhoid Fever.—A characteristic parasite was discovered in 1880 by *Eberth*[31] in the implicated viscera in typhoid. Similar observations were afterwards made by *Klebs* and *Eppinger*,[32] and these have been confirmed by the researches of *R. Koch*, *Meyer*, and *Friedländer*, and more recently by *Gaffky* and very many others.[33]

Gaffky describes the fungus as consisting of rods, in length equal to

Fig. 54.—Bacilli of Typhoid (pure cultivation).

one-third the diameter of a red blood-corpuscle, and occasionally forming threads of greater length by the aggregation of several segments. They are about three times as long as they are broad, and rounded off at the extremities. Spores are sometimes to be seen within the rods. They stain best in a concentrated watery solution of methylene blue, and *Löffler's* process (p. 28) is the most appropriate to the purpose.

Gaffky[34] has ascertained certain facts connected with the life-history of the typhoid bacillus. It develops readily in a medium of nutrient gelatine. In a preparation of this kind the cultivations make their appearance after the lapse of twenty-four hours. When examined with a medium power of the microscope, they seem to be yellowish, and they do not render the gelatine fluid. Rods and threads appear, and they are endowed with an evident and peculiar motion. The parasite develops on prepared potato. The colonies can be distinguished with difficulty by the naked eye. When cultivated in a

medium of prepared potato at a temperature of 37° C., spores begin to form after three or four days. According to *Birch-Hirschfeld*,[35] solitary and segmented spores are to be seen, the first in hanging-drop cultivations, and the latter when cultivated in an incubator. This observer recommends that the nutrient medium be stained with phloxin-red or benzo-purpurin, by means of which the spores are deeply coloured. There is much doubt as to the true character of these so-called spores (*Buchner*, *Pfuhl*).[36] The fungi are easily cultivated in hanging drops of sterilised broth.

The bacillus occurs also in the stools of typhoid patients; but on account of the vast number of micro-organisms constantly to be found in the dejections, it is impossible to recognise it with the aid of the microscope alone. Neither does it possess, like the bacillus of tubercle, any characteristic staining properties. For its complete recognition, therefore, we must resort to *Koch's* methods for obtaining pure cultivations, and separate it from the fæcal substances. *Pfeiffer*[37] was the first to do this, and he made use of the nutrient agar-agar plate. A useful adjunct is the employment of a 0.2 per cent. solution of carbol-gelatine, after the manner of *Chantemesse* and *Vidal*.[38]

It is difficult to effect the separate cultivation of typhoid bacillus derived from the fæces in nutrient gelatine, because the discharges contain other micro-organisms (hay bacillus), which liquefy that medium before the colonies of typhoid bacilli appear in it.

When the micro-organism under discussion has been experimentally communicated to animals, it causes them to manifest the symptoms of typhoid; and the researches of *E. Fränkel*, *M. Simmonds*, and *C. Seitz*,[39] seem to leave no doubt as to its pathogenic character. *Beumer* and *Peiper*,[40] on the other hand, have come to a different conclusion. Bearing in mind *Brieger's* discovery of animal alkaloids (*ptomaines*) as a product of bacillary life, it seems probable that such play an important part in connection with typhoid, and this may well explain the controverted results obtained in the experiments upon animals reported by *Fränkel*, *Simmonds*, and others.

Finally, it may be noticed that many recent investigations tend to prove the possibility of typhoid bacilli being disseminated by means of drinking-water and by milk.[41]

4. Bacillus of Tubercle.—*Lichtheim*[42] and other observers have found tubercle bacillus in the stools in cases of tubercular ulceration of the intestine. For its recognition there, the methods are employed which have already been described in connection with the sputum (*vide* p. 75).

The detection of this bacillus in the fæces invariably implies the

existence of tubercle, but not necessarily tubercle of the intestines, since it may be derived from sputa which have been swallowed. Still, in cases where it has been repeatedly found in the stools, and where it occurs in great profusion, so as to resemble pure cultivations (*vide* fig. 89), and especially where the other symptoms of tubercular ulceration of the intestine (purulent discharges, &c.) are present, the diagnosis is established on the firmest basis.

B. ANIMAL PARASITES.

1. **Protozoa.**—Adopting the classification of *Leuckart*,[43] these include *Rhizopoda*, *Sporozoa*, and *Infusoria*.

1. Rhizopoda.

(*a.*) **Monadines.**—*Nothnagel*[44] found these organisms repeatedly in the stools of consumptive and typhoid patients, and of persons suffering from heart-disease. They were always dead unless the stools were examined directly upon being passed, and then appeared as more or less circular bodies of various sizes (fig. 55, *f*).

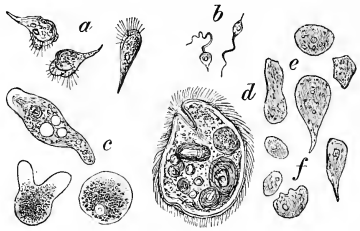


FIG. 55.

a. Trichomonas intestinalis.
b. Cercomonas intestinalis (*Davaine*).
c. Amœba coli.
d. Paramæcium coli.
e. Monadines, living.
f. Monadines, dead.

The still living monadines are pear-shaped, and usually possess a long pointed process, which moves about rapidly (fig. 55, *e*). According to *Nothnagel*, they have no pathological significance. *Grassi*[45] found similar bodies in the stools of a patient suffering from entero colitis. Bodies altogether resembling these, but not as yet identified with them, occur in the discharges of infants and children (see *v. Jaksch*).[46]

(*b.*) **Amœba coli.**—*Lösch*[47] has described certain cellular bodies of large size which he found in the fæces in a case of intestinal ulcer of a quasi-tubercular character. These bodies were contractile, and some of them, of circular form, had a diameter of 20–35 μ. They consisted of hyaline and coarsely granular protoplasm, with a round nucleus and hyaline vesicle, but no distinct cell-wall (fig. 55, *c*).

Similar organisms have been found in the intestine by *Lambl*,[48] and *Kartulis*[49] recently reports having seen amœba-like organisms in the stools of Egyptian natives suffering from dysentery and chronic enteritis.

2. **Sporozoa.**—Again reverting to *Leuckart's* classification, the sporozoa which chiefly concern us here are the oval psoro-spermia, the group to which belongs the coccidia which infest the intestinal tract of man (*Dressler*, *Gubler*, *Kjellberg*, *Eimer*).[50] They appear in the fæces as elliptical forms, 0.022 mm. long, furnished with a thin membrane, and enclosing within their substance a number of granular nuclei, arranged for the most part in groups. They are to be found in great numbers. Their favourite seat is the intestinal mucous membrane, where they burrow, doing much damage to its structure. For this reason *Leuckart* appropriately names the parasite Coccidium perforans.

3. **Infusoria.**—1. **Cercomonas intestinalis.**—This protozoon was first

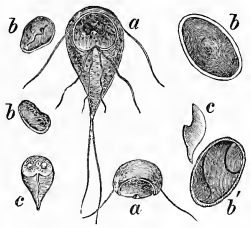


Fig. 56.—Cercomonads from the Stool.

a, Megastoma entericum (*Grassi*).
b, *b'*, Encysted forms of Cercomonas intestinalis.
c, Cercomonas intestinalis after loss of its tentacles (*Lambl*).

found by *Lambl*[51] in the jelly-like mucous discharges of children, and it was afterwards observed by *Davaine*, *Marchand*, and *Zunker*.[52] It is of piriform shape, clearly nucleated, and furnished with eight tentacles of varying length (see fig. 56, *a*). Davaine discovered it in cholera, Marchand in typhoid, and Zunker in nine cases of diarrhœa. It would appear from these facts that the Cercomonas intestinalis is apt to thrive in a gut which is already diseased, and when present tends to cause severe diarrhœa. The observations of Zunker especially strengthen this conclusion.

The **Megastoma entericum** lately described by *Grassi* is almost certainly identical with Lambl's Cercomonas.[53] *Perroncito* has observed the encysted forms of the parasite in the intestine (fig. 56, *b*)—observations confirmed by the author in the case of children.[54] According to *Grassi* and *Schewiakoff*,[55] its presence is associated with anæmia and

diarrhœa, and it also impedes absorption from the intestine by its effect upon the lining cells of the mucous membrane on which it lies.

Other cercomonads have been seen in the same situation (*Davaine*), (see fig. 55, *b*).

2. **Trichomonas intestinalis.**—This is a pear-shaped organism, somewhat larger than Cercomonas intestinalis, and distinguished from it by bearing a ciliated disc at one extremity (fig. 55, *a*). It has been obtained from the intestine by *Marchand* and *Zunker*.

3. **Paramæcium coli** (*Balantidium coli*).—An entozoon first found by *Malmsten*[56] in the discharges of diarrhœa, and afterwards observed by *Stieda, Graziadei*, and *Perroncito*.[57] It is oval, 0.1 mm. long, and covered with cilia, which are planted more thickly around the buccal (?) orifice. The anterior extremity is smaller than the posterior, and the opening (anal) in this situation is but sparingly provided with cilia. The abdominal surface is less arched than the dorsal. Internally it is furnished with a nucleus and two contractile vesicles, and frequently contains amylum particles and fatty granules. Its presence appears to be associated with diarrhœa, but it has no other clinical significance.

In addition to the varieties mentioned here, other infusoria are occasionally present in the intestine (*V. Jaksch*).[58]

2. Vermes.—The investigation of the fæces for intestinal worms has become of late years a matter of special interest to physicians, because experience daily teaches us that, even in temperate climates, the alimentary canal is apt to be beset with certain parasites of this class, which must be reckoned amongst the most formidable pests of mankind; and it often happens that an accurate diagnosis of their nature can alone enable the physician to adopt intelligent methods for their removal, and so, it may be, to save his patient's life.

Class I.—Platodes.

(*a.*) **Cestodes.**—The following tapeworms concern us here:—

1. Tænia saginata (mediocanellata).
2. Tænia solium.
3. Tænia nana.
4. Tænia flavopunctata.
5. Tænia cucumerina (elliptica).
6. Bothriocephalus latus.

1. **Tænia saginata** (*mediocanellata*).[59]—This parasite is longer than the Tænia solium, attaining to four or five metres, and its segments are also longer. The head is surrounded with four large and usually black-pigmented suckers, but is not provided with a rostellum, and is without a circle of hooklets. The segments increase in length more gradually than in Tænia solium, and are commonly pigmented.

The uterus is very much branched, and the genital pore is situated

at the side of the proglottis. The eggs resemble those of Tænia solium, but are more oblong, and exhibit the primordial yolk membrane. Hooklets are not discernible in the embryo (fig. 57).

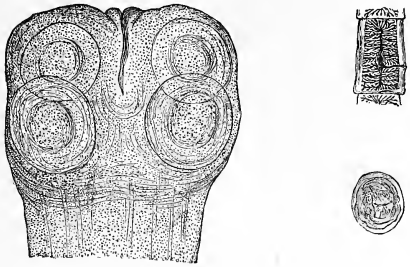


Fig. 57.—Tænia saginata: Head; Proglottis; Egg.

2. Tænia solium.[60]—The Tænia solium may measure upwards of two or three metres. Its head is quadrilateral, about as large as a pin's-head ($\frac{1}{45}$ to $\frac{1}{35}$ of an inch), and dark in colour. This is succeeded by a delicate, thread-like neck, about an inch in length, and unjointed. The segments, or proglottides, which form the rest of the body, are short and relatively broad near the neck; but as they increase in size

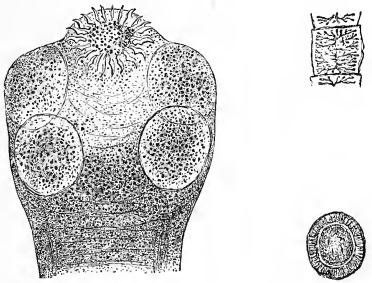


Fig. 58.—Tænia solium: Head; Proglottis; Egg.

this relation ceases, and still growing in both dimensions, their quadrilateral form becomes evident about three feet from the head. Their average length is from 9 to 10 mm., and their breadth 6 or 7 mm.

Under the microscope the head is seen to present four prominent suctorial discs, usually pigmented, and between them a rounded elevation or rostellum, which is surrounded with about twenty-six hooklets of different sizes.

The sexual apparatus first becomes visible about a foot from the head (*Bristowe*). The uterus is but little branched, and the genital pores are situated somewhat behind the middle of each segment.

The eggs are oval in shape, about 0.03 mm. in diameter and 0.036 mm. long, and surrounded with a thick shell, which is radially striated. When the eggs are mature, they may be seen to contain embryos furnished with hooklets (fig. 58).

3. Tænia nana.[61]—This parasite averages from 10 to 15 mm. in length, and its greatest breadth is about 0.5 mm. It has a globular head 0.3 mm. in diameter, furnished with four circular suckers, and a rostellum 0.06 mm. long, carrying twenty-two to twenty-four hooklets at its anterior extremity, which is rounded off. The rostellum can be protruded to a considerable distance from the head or entirely withdrawn within it. In the latter position it has the form of an hour-glass. The body is attenuated in its anterior third, but proceeding backwards grows quickly in bulk. The segments are short, and towards the end of the

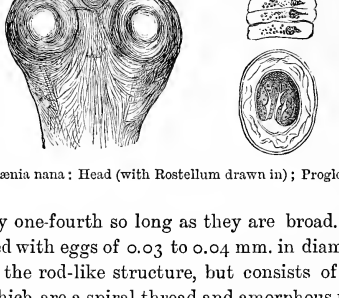


FIG. 59.—Tænia nana: Head (with Rostellum drawn in); Proglottis; Egg.

body are scarcely one-fourth so long as they are broad. The uterus is oblong and loaded with eggs of 0.03 to 0.04 mm. in diameter. The shell does not exhibit the rod-like structure, but consists of a double membrane, within which are a spiral thread and amorphous matter, in which granules are embedded. In the interior of the egg may be seen the embryo, already provided with five or six hooklets (fig. 59). The parasite may be present in great numbers in the intestine, and is apt to produce severe nervous symptoms, such as epileptic seizures, insensibility and mental derangement, and melancholia (*Grassi, Comini*).[62]

Recent observations go to prove that the Tænia nana is very widely distributed. It especially attacks children and young persons. It was first discovered by *Bilharz* in Egypt, and since then has been recognised as of common occurrence in Italy and Sicily.[63] *Ranson* has published an account which goes to prove that it was observed in

England so long ago as 1856.[64] According to Grassi, Tænia nana is in Sicily the commonest parasite. The number present in a single individual may reach 4000–5000.

4. Tænia flavopunctata.—This bears a close resemblance to Tænia nana, but it is of greater length (10 to 12 mm.), has no rostellum, and only two suckers. The eggs are like those of Tænia solium (*Parona*), and larger than those of Tænia nana. The occurrence of the parasite in man rests upon the testimony of *Leidy* and *Parona*,[65] but is disputed by others; and *Grassi*[66] identifies it with Tænia leptocephala, which has hitherto been found only in certain species of mice and rats (*Mus decumanus* and *Mus rattus*).

5. Tænia cucumerina (elliptica).—This is a worm which averages 18–25 cm. in length. The head is situated at the thinner end, and has some sixty hooklets irregularly disposed around the rostellum, which is a coarse club-like process when projected. The first forty proglottides are of small size, but increasing as they proceed backwards

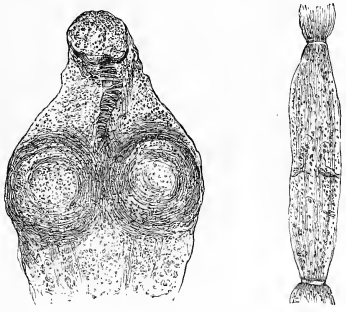


FIG. 60.—Tænia cucumerina: Head; Proglottis.

both in length and breadth, and especially in the former dimension, measure at the extremity 6 to 8 mm. by 2 mm. in breadth. The mature segments are of a reddish colour, and readily separate from those nearer to the head. The eggs, when shed, have a diameter of 0.05 mm., and contain an embyro furnished with hooklets.

The parasite is not at all rare amongst human beings;[67] it especially infests children, and is acquired by them from dogs.

It is sufficient merely to mention here the rarer forms of tape-worm known to be present in the human intestine. These are Tænia madagascariensis (*Grenet*[68]) and Tænia leptocephala (*Grassi*), which possibly is the same as Tænia nana.[69]

6. Bothriocephalus latus.[70]—This worm attains a length of 5–8 metres. The head is ovoid, and 2 mm. long by 1 mm. broad. It is cleft, and provided with two lateral suckers, placed on either side of the

middle line. It has no hooklets. The proglottides are at first short and small. They increase in breadth as they proceed, and towards the end of the parasite approach the square form.

The uterus of mature proglottides containing eggs exhibits a retiform arrangement, and appears superficially as a small rosette. This uterine rosette is characteristic of Bothriocephalus latus.

The eggs are oval, and measure 0.07 mm. by 0.045 mm. They are covered with a brown shell, and open by a small lid at one end. They contain centrally-transparent masses of protoplasm of uniform size. Bothriocephalus latus has recently acquired an increased clinical significance, inasmuch as it has been found frequently by different observers in conjunction with the symptoms of pernicious anæmia.[71]

Other varieties of this parasite have been observed, as the B. cordatus in Greenland,[72] and the B. liguloides in Japan and China. The latter infests the sub-peritoneal tissue, and especially that of the lumbar region in man.[73]

The presence of one of the tape-worms here described is made evident—apart from clinical symptoms, which do not concern us here—by the discovery of proglottides in the stools.

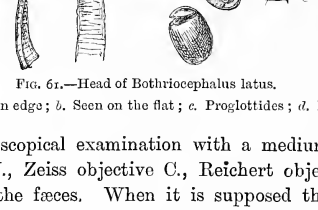


FIG. 61.—Head of Bothriocephalus latus.
a. Seen on edge; *b*. Seen on the flat; *c*. Proglottides; *d*. Eggs.

A careful microscopical examination with a medium power (Hartnack objective IV., Zeiss objective C., Reichert objective IV.) may show the eggs in the fæces. When it is supposed that a patient is suffering from tape-worm, whilst yet no evidence of the parasite can be derived from a careful inspection of the stools, it is well to mix these with water, which is constantly poured off and renewed until the greater part of the fæcal mass has been dissolved. An examination of the sediment will then inevitably reveal the eggs, if the suspicion be well founded. To determine to what form of tape-worm a particular proglottis belongs, the best proceeding is to examine the specimen, mounted in glycerine, with a low magnifying power; and the same thing may be done if a head be obtained.[74]

Cysts of echinococcus and hooklets may be found in the fæces when a hydatid cyst has burst into the intestine. *Heller*[75] was able by the discovery of such a cyst to diagnose a doubtful liver complaint as one of hydatids.

(*b.*) **Trematodes.**[76]—The varieties of distomata, D. hepaticum and D. lanceolatum, have been found in rare instances in the intestine or biliary passages of man.

1. Distoma hepaticum.—This is a leaf-shaped worm, measuring 28 mm. by 12 mm. The head is short, and furnished with a sucker. There is another sucker on the ventral aspect, and between the two the genital pore is situated. The latter leads to a uterus, which is convoluted like a ball of wool.

The eggs are oval, 0.13 mm. long and 0.08 mm. broad, brown in colour, and covered with a shell consisting of two layers; one end is broader than the other, and opens by a small lid. *Biermer*, *Bostroem*, and *Baelz*,[77] have described these parasites as occurring in human beings.

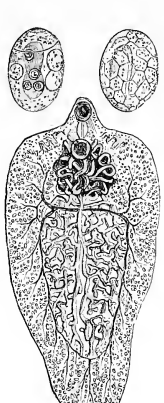


FIG. 62.—Distoma hepaticum.

2. Distoma lanceolatum.—This entozoon is from 8 to 9 mm. long, and 2–3.5 mm. broad. It is lance-shaped, as its name implies, and pointed at either extremity, but more so in front than behind. In other respects its form resembles that of Distoma hepaticum.

The eggs are 0.04 mm. long, and 0.03 mm. broad, and they contain the mature embryo. *Bizzozero*[78] maintains that when the worm is present in the intestine, its eggs are to be found in the stools. The observations of Baelz (see *ante*) corroborate this view. *Perroncito*[79] found the eggs of this fluke in the case of persons infested by anchylostoma.

The last two forms of parasites but rarely occur in man, and con-

sequently neither they nor their ova are often to be seen in the fæces. Moreover, they very seldom cause serious symptoms.

3. Distoma Rathonisi.[80]—This distoma was described by *Rathonis*, who observed the first specimen in a Chinese woman, thirty-seven years of age. The host suffered from severe pains, referred to the liver. The parasite resembles D. hepaticum, but is larger (25 mm.); the ramifications of the alimentary canal are less complex, especially towards the hinder end.

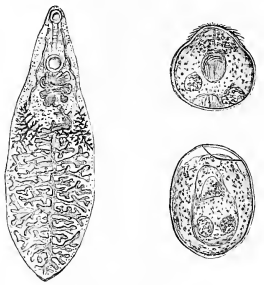


FIG. 63.—Distoma lanceolatum.

CLASS II.—ANNELIDES.

1. *Order Nematodes* (*Round Worms*).

a. Family Ascarides.[81]

1. Ascaris lumbricoides (common round worm).—This is a cylindrical worm, of some size, with a body that tapers from before backwards. The male is 250 mm. and the female 400 mm. long. The head, which is distinct from the body, consists of three conical prominences (lips) furnished with tactile papillæ and minute teeth. The caudal process of the male is folded hook-like on the abdominal surface, and is provided with papillæ. In the female, the vulva lies deeply behind the anterior third of the body. The eggs are nearly round, and brownish-yellow in colour. Their diameter is 0.06–0.07 mm. In the fresh state they are covered externally with an albuminous layer, and beneath this is a tough shell, which in turn encloses the very granular contents.

The Ascaris lumbricoides infests the small intestine in man, and it appears to be common to all climates. It occurs also in cattle and in sheep. It has no special medical interest; but it is thought by *Lutz*[82] to cause spasm and tympanitis, and to impede nutrition in children. *Kartulis*[83] records a case of death in a man following directly upon an invasion of the liver by ascarides. Severe nervous symptoms also, as

amaurosis, strabismus, and evidence of meningitis,[84] have been found to accompany their presence in great abundance.

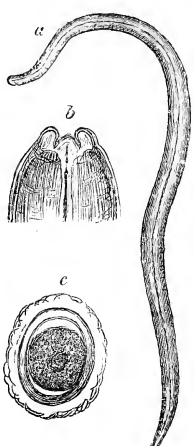


FIG. 64.—Ascaris lumbricoides.
a. The Worm; *b*. Head; *c*. Egg.

2. **Ascaris mystax** (round worm of the cat).—This worm closely resembles the preceding, but is smaller, and is readily distinguished

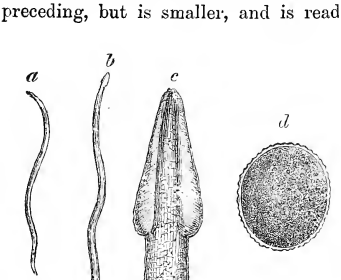


FIG. 65.—Ascaris mystax.
a. Male; *b*. Female; *c*. Head; *d*. Egg.

from it by the shape of its head, which is pointed, and bears two lateral wing-shaped processes. The male is 45–60 mm. long, and the female

110–120 mm. The eggs are globular, larger than those of A. lumbricoides, and covered with a dimpled shell (fig. 65).

3. **Oxyuris vermicularis** (common thread-worm or teat-worm).—The female is 10 mm. in length, and exhibits two fully-developed uteri, which extend symmetrically backwards from the vaginal orifice. The male is rather less than half the length of the female, and its tail is provided with six pairs of papillæ. The head of both sexes is similar. It displays a remarkable cuticular enlargement and small prominent lips.

The eggs are irregularly oval, and measure $\frac{1}{490}$ (0.05 mm.) by $\frac{1}{1100}$ (0.02–0.03 mm.) inch. The shell is membranous, and consists of two or three laminæ. Its contents are coarsely granular. The eggs often

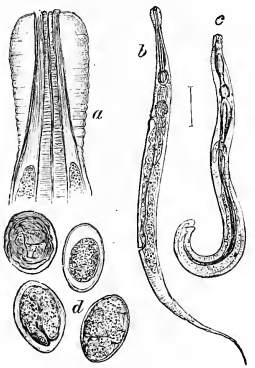


Fig. 66.—Oxyuris vermicularis.
a. Head; *b*. Male; *c*. Female; *d*. Eggs.

contain an embryo with an indistinct alimentary canal and a tail equal to half the entire length. The presence of the parasite is attended with uncomfortable sensations, among which itching in the situation of the anus is prominent.[85]

β. Family Strongylides (*Leuckart*).[86]—To this family belongs one of the most important and formidable of the parasites which infest the human intestine. This is—

Anchylostoma duodenale (Dochmius duodenalis, Strongylus duodenalis).—It was formerly believed that this worm occurred only in the tropics and in certain districts of Italy.[87] Of late years, numerous observations in Italy (*Perroncito* and others[88]), Germany, Switzerland (*Menche* and others[89]), and Belgium[90] have made it clear, together with

the older researches,[91] that the inhabitants of temperate regions are not free from its attacks.[92]

The anchylostoma is cylindrical in form. The male measures 8 to 12 mm. in length, the female 10 to 18 mm. The anterior extremity is pointed, and reflected towards the dorsal surface. The oral orifice is armed with four claw-like teeth. The caudal extremity of the male expands into a pouch with three flaps; that of the female is pointed and conical; the vulva is situated behind the middle third of the body.

The eggs are smooth and oval, measure 0.05 to 0.06 mm., and usually contain two or three large daughter-cells. The embryos develop rapidly outside the human body. In stools which contain eggs, the embryo

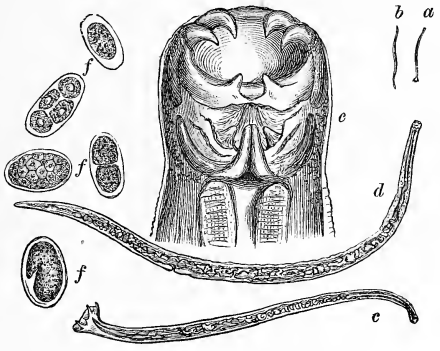


FIG. 67.—Anchylostoma duodenale.

a. Male (natural size).
b. Female (natural size).
c. Male (magnified).
d. Female (magnified).
e. Head.
f. Eggs.

may be seen and observed after the lapse of twenty-four to forty-eight hours.

Except when anthelmintics have been administered, the eggs are the only signs of the parasite to be found in the discharges, and it is consequently of the utmost importance to be familiar with their appearance. The accompanying figure shows this at various stages of their development (fig. 67, *f*).

The presence of anchylostoma is to be suspected in all cases where a severe form of anæmia occurs in labourers (and especially in brick-burners, miners, and tunnel-borers), without any obvious and sufficient cause. It is, however, necessary to bear in mind that Bothriocephalus latus produces similar symptoms (p. 146). Examination of the stools will then afford the means of diagnosis. If the intestines contain

anchylostoma, the characteristic eggs with their large blastomeres will be seen. If any uncertainty as to their nature should persist, the fæcal substances containing them should be allowed to remain for some days in a warm place, and again examined microscopically. The process of segmentation of the egg will then be seen to have advanced, and fully developed embryos will be visible here and there. The administration of anthelmintics, and especially of the æthereal extract of male-fern, will cause the expulsion of the mature worm, and an inspection of the resulting discharges cannot fail to establish the diagnosis.

The character of the stools in this affection varies greatly. Diarrhœa is usually present, and blood is frequently passed. But it may happen that the discharges are altogether normal. They have been known to

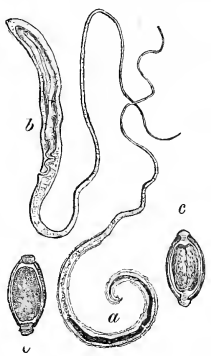


FIG. 68.—Trichocephalus dispar.
a. Male; *b*. Female; *c*. Eggs.

contain great quantities of Charcot-Leyden crystals (*Leichtenstern*, see *ante*).

7. *Family Trichotrachelides* (*Leuckart*).[93]

1. Trichocephalus dispar (whip-worm).—This worm has a whip-like form, consisting of a short, stout hinder-part, and a long, spiral, filiform process anteriorly. The male is 40 mm., and the female 50 mm. long. The short hinder part measures 1 mm. in thickness (fig. 68, *a*, *b*).

The eggs, which are occasionally to be found in the fæces, are brown in colour, 0.05–0.06 mm. long and 0.02 mm. broad. The shell consists of two layers and is flattened at either end, where it is furnished with

a small lid, formed of a glossy substance. The yolk is very granular (fig. 68, *c*). According to *Erni*[94] and others, this parasite, together with anchylostoma and an insect-larva, produces the beri-beri disease which is endemic in Sumatra. This view is, however, opposed by other writers (*Scheube*, *Scheffer*).

2. **Trichina spiralis.**[95]—Trichina occurs in two different forms in the human body, according as its habitat is the muscular tissue or the intestine. It is with the trichina of the intestine that we have to do here, since this form alone is found, though rarely, in the fæces. The male is 1.5 mm. in length, and the female 3 mm. The former has four prominent papillæ situated between the conical protuberances at

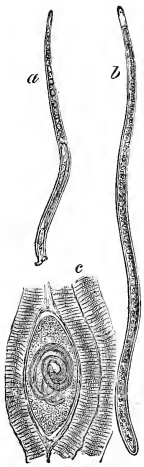


FIG. 69.—Trichinæ.

a. Male and *b*. Female Intestinal Trichinæ; *c*. Muscle Trichina.

the extremity. The sexual organs of the female consist of a tubular ovary, which is placed at the hinder part of the body, and a tubular uterus, with which the ovary communicates in front (fig. 69). Impregnation takes place in the intestine. The eggs develop into embryos while still within the uterus, and the newly-born parasite almost immediately perforates the gut, and becomes imbedded in the muscles of its host.

Of their own accord these worms rarely pass with the stools. But in any case where there is reason to believe that trichinous meat has been eaten and trichinosis apprehended, an anthelmintic may be given,

and the detection of intestinal trichina in the fæces will establish the diagnosis in a very early stage of the affection.

δ. *Rhabdonema Strongyloides* (*Leuckart*).—Certain nematode worms have been discovered in the stools in cases of Cochin-China diarrhœa (*Normann, Bavay, Seifert*).[96] They occur commonly in conjunction with anchylostoma (*Grassi, Parrona, Perroncito*).[97] It was formerly thought that there were two distinct parasites of this kind, viz., Anguillula intestinalis and Anguillula stercoralis; but more recent observations by *Leuckart* and *Grassi*[98] have shown that the latter is only an intermediate form in the development of A. intestinalis. A complete knowledge of the subject, however, implies an acquaintance

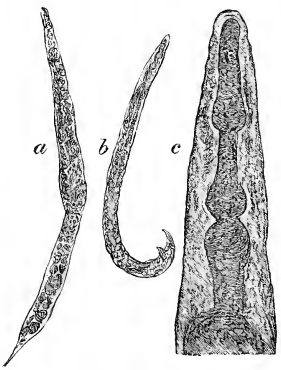


FIG. 70.—Anguillula stercoralis.
a. Female; *b*. Male; *c*. Head.

with this form, as it may be confounded with other helminthidæ. The life-history, according to Grassi, is as follows:—

The anguillula which infests the human intestine deposits eggs, from which the young are produced as embryos or larvæ. These are discharged with the stools. As found there, they already exhibit sexual maturity (*Anguillula stercoralis*), and produce embryos, which undergo no further change in the human system. The body of this worm is round, and shows faint traces of transverse striation. The head is in the form of a blunted cone (fig. 70) and sessile upon the body. It is furnished with two lateral jaws, each bearing a pair of teeth. The male is 0.88 and the female 1.22 mm. long. *Anguillula intestinalis* measures 2.25 mm. in length and 0.04 mm. in thickness at its middle. It has a triangular mouth closed by three small lips. The vulva lies at the junction of the middle with the posterior third. Its habitat is

the small intestine. The eggs resemble those of Anchylostoma duodenale, but are longer, more elliptical, and pointed at the poles. In recent stools the larvæ alone are to be seen. It is doubtful whether the parasite is directly mischievous; but its constant association with anchylostoma, and the readiness with which the two may be confounded, render its recognition a matter of consequence.

3. Insects.—It requires to be noticed that insect-larvæ infest the stools. *Joseph* [99] has reported a number of species, which are for the most part taken into the intestine with food (cheese, meat), and give rise to a variety of morbid symptoms, as colicky pains, vomiting, &c. (see p. 111). Special mention may be made of the immature cheese-maggot (Piophila casei) and Drosophila melanogastra, which are derived from curdled milk, and may attain the chrysalis form in the intestine before they are discharged per rectum. Of other species are the larvæ of three varieties of Homalomyia, of Hydrothæa meteorica, Cyrtoneura stabulans, Calliphora erythrocephala, Pollenia rudis, Lucilia cæsar and regina, Sarcophaga hæmorrhoidalis and hæmatodes, and Eristalis arbustorum, all of which are apt to occur. *Rembold* and *Lampa* [100] have also observed in the stools certain lancet-shaped bodies, 8 mm. long, which are covered with hair and indented on the surface, and these have been identified by *V. Graff* as the larvæ of Anthomyiæ. (See Appendix XIV.)

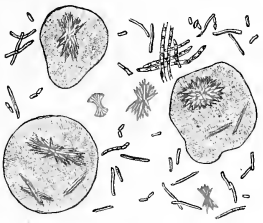


Fig. 71.—Hæmatoidin Crystals from the Fæces.

4. CRYSTALS.—Crystalline bodies are a common constituent of the fæces, and in some cases are to be found in great quantities. They may be organic or inorganic.

1. Charcot-Leyden Crystals.—These bodies, of which an adequate description has already been given in connection with the *Blood* (p. 21) and the *Sputum* (p. 81 and fig. 46), are comparatively rarely to be seen in the fæces. *Nothnagel* has met with them in typhoid fever, and *Leichtenstein* [101] in phthisis and anchylostomiasis. Their presence, however, has no pathological significance.

2. Hæmatoidin Crystals.—It is surprising that so little attention has been paid to this subject. *Uffelmann*,[102] indeed, remarks that crystals

of hæmatoidin are sometimes present in the discharges of infants at the breast; but apart from this notice the matter has been passed by in silence. The author has found these crystals often enough in the fæces, especially in chronic intestinal catarrh from over-eating, and in many instances in which blood had been discharged into the intestine some time (several days) before the stools were passed. They usually exhibit an ill-marked crystalline structure. Particularly good specimens were seen in the stools of a patient suffering from nephritis. The crystals are sometimes free, and sometimes enclosed in masses of a shining substance which resembles mucin (fig. 71).

3. **Cholesterin.**—This substance enters normally into the constitution of the fæces, and can always be obtained from them.

It seldom appears in its crystalline form (fig. 106), according to Nothnagel, and the statement is undoubtedly correct. (For the microscopical and chemical character of cholesterin, *vide infra* and chapters on *Sputum* and *Urine*.)

4. **Fat Crystals.**—*Nothnagel*, in his well-known monograph, states that fatty substances occur in the fæces in the form of needles.

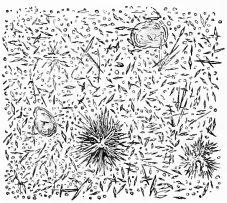


Fig. 72.—Fæces of Jaundice.

Gerhardt[103] found quite an enormous quantity of organic crystals in the stools of jaundice (fig. 72). He was himself of opinion that such bodies consisted of tyrosin; but one of his pupils, named *Oesterlein*,[104] who pursued the investigation farther under *Gerhardt's* auspices, came to the conclusion that chemically they were lime and magnesia salts of the higher fatty acids, and that, therefore, soaps of lime and magnesia bases form a part of such discharges. According to *Stadelmann*,[105] they are sodium soaps. The author, however, can add his own testimony to Gerhardt's statement, for he has repeatedly seen great quantities of crystals arranged in clusters in acholic stools; and is disposed, on the grounds of separate investigation, and especially of the ascertained character of similar bodies occurring in other excretions (see chapter on *Urine*), to believe that such crystals do not consist of tyrosin, but rather of a combination of the alkaline earths with the higher fatty acids.

Uffelmann,[106] who had already observed these crystals in the discharges of infants, arrived independently at the conclusion that they could not consist of tyrosin. According to *Fr. Müller*,[107] their presence indicates an impediment to the absorption of fat from the intestines.

In cases of jaundice, so frequent among children, such crystals are profusely present in the stools, and they are normally found in those of infants during lactation (*V. Jaksch*).

5. Oxalate and other Organic Salts of Lime.—Oxalate of lime is a sufficiently common constituent of the fæces. According to *Nothnagel*, it is always derived from the food. It is most abundant after a vegetable diet, and when manifested in considerable quantity, the discharges also contain abundant débris of plant-tissues.

Uffelmann[108] asserts that crystals of lactate of lime occur in the discharges of children as sheaves of radiating needles. Other organic salts of lime, as the acetic and butyric acid salts, have been observed in the discharges of persons suffering from acute gastric and intestinal catarrh (*V. Jaksch*).[109]

6. Carbonate of Lime occurs rarely in the stools as amorphous particles and dumb-bell figures (fig. 105).

7. Sulphate of Calcium.—This salt is very seldom present in the stools. It can be obtained, however, by the action of sulphuric acid on the fæces, which shows that other lime salts are present. Its form presents the same variety here as in the urine (fig. 97).

8. Phosphate of Lime.—This substance crystallises in stout or elongated wedges, grouped together so as to form larger or smaller gland-like masses (fig. 96). The presence of such crystals in the fæces is without pathological significance.

Other salts of lime are occasionally met with. They are impregnated with bile-colouring matters, and deeply stained a yellow colour.

9. Triple Phosphate.—The phosphate of ammonia and magnesia occurs sometimes as well-formed coffin-lid crystals (fig. 48, *k*), sometimes in ill-defined crystalline masses, and very rarely in the elder-leaf arrangement (fig. 102).

Perfect crystals are most commonly to be found in fluid motions and in the mucus which adheres sometimes to the fæces, whether liquid or solid. Fragments of coffin-lid crystals alone may be visible, and these often exhibiting fissures and flaws, or there may only be mere splinters (*Nothnagel*). It is notable that these crystals seldom take up bile pigment. By their chemical constitution they may be readily recognised; and they dissolve easily, as has been already mentioned, in acetic acid (p. 83).

10. Sulphide of Bismuth Crystals.—When preparations of bismuth have been taken internally, the stools may be found to contain crystals

which bear a remarkable resemblance to those of chloride of hæmatin (hæmin). They consist of sulphide of bismuth, as may readily be shown by a comparison with the bodies formed when nitrate of bismuth is added to ammonium sulphide.

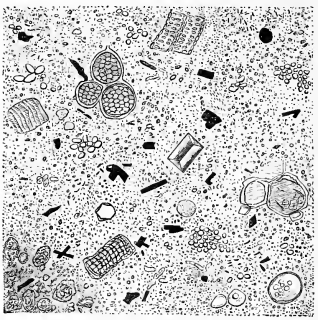


Fig. 73.—Sulphide of Bismuth Crystals in the Fæces.

III. CHEMICAL EXAMINATION OF THE FÆCES.—In striking contrast with the valuable information to be derived from the naked-eye and microscopic investigation of the fæces is the very slight assistance which the chemical examination of those discharges lends to the purposes of diagnosis.

A. **Organic Substances.**

1. Mucin.—Mucin, as *Hoppe-Seyler*[110] has said, may be looked upon as the basis of the fæcal substances, a statement which can be confirmed by the author. For the detection of mucin in the stools the following process may be recommended:—The fæces are stirred up with water, their own bulk of lime-water added, and the mixture allowed to stand for some hours. It is then filtered, and the filtrate tested with acetic acid for mucin (see chapter on *Urine*).

2. Albumin.—The presence of albumin may be shown as follows:—The fæces are mixed with a large quantity of water, to which a trace of acetic acid has been added, and a watery extract is thus made. This is repeatedly filtered, and the filtrate may be submitted to the tests for albumin mentioned in the chapter on *Urine* (*q. v.*). Under ordinary circumstances no proteid reactions can be obtained, but albumin occurs in considerable quantity in the stools of typhoid and diarrhœa. In one instance, much serum-albumin was found in the discharges of a chlorotic woman, who passed pale stools almost devoid of bile; and once again in the case of a patient whose stools were deficient in bile, but who was not jaundiced (*V. Jaksch*).

3. Peptone.—For the detection of peptone, the fæces should be mixed with water so as to form a thin pulpy fluid, then boiled, and filtered while still hot. The filtrate will be clear or slightly tinged with red. When it has cooled, it may be tested with acetic acid and ferrocyanide of potassium for albumin. It usually happens that the liquid becomes a little turbid when the acetic acid is added (mucin), but the turbidity does not extend on the addition of ferrocyanide of potassium. When this is so, the mucin may be precipitated with acetate of lead, the filtrate tested in the manner afterwards to be described (chapter on *Urine*) with phosphotungstic acid, and the fluid which finally remains may be submitted to the biuret test. Should it happen that the fluid after boiling contains albumin as shown by the acetic acid and ferrocyanide of potassium test, this substance must be removed by combination with ferric acetate (see chapter on *Urine*), and the remainder of the process conducted as above.

The author has never found peptone in healthy fæces, but has met with it repeatedly in disease. He has collected the records of some fifty or sixty cases bearing upon this matter, and the results which were obtained depend upon seventy or eighty separate analyses.

Out of seven cases of typhoid, peptone occurred in the liquid stools in large quantities in five. Its presence remained doubtful in one, and it was absent from the discharges of the seventh.

It was present in all cases where the stools contained pus, as in dysentery (two cases), tubercular ulceration of the intestine (three cases), and suppurative peritonitis discharging pus into the intestine (one case).

In hepatic affections, the character of the fæces in this respect was very inconstant. In a series of cases of catarrhal jaundice with more or less acholic stools, no peptone was to be found; whilst, on the other hand, the thin, non-purulent discharges from a patient with syphilitic inflammation of the liver exhibited it in considerable quantity. It occurred plentifully in certain cases of atrophic cirrhosis and of carcinoma of the liver.

The acholic stools of jaundice were generally rich in peptone, but the results of analysis in these cases show much variety (*v. supra*).

4. Urea.—The presence of urea may be best ascertained by the method previously described (p. 46). For the estimation of metabolism, it is necessary to ascertain the total quantity of nitrogenous substances contained in the fæces. To do this, the stools should be treated with dilute acid (to prevent the evaporation of ammonia), and dried. The usual method for the analysis of organic compounds will then serve to determine the proportion of nitrogenous bodies present.

5. Carbohydrates.—Various carbohydrates occur in the fæces.

Of these, starch is the most prominent. It may be recognised at any time by the aid of the microscope. *Hoppe-Seyler*[111] asserts that grape-sugar and certain gummy carbohydrates may be contained in the stools.

To ascertain the presence of these substances, the fæces must be boiled with water, filtered, and the filtrate partially evaporated on a water-bath. One part of the fluid may then be tested with Trommer's or the phenyl-hydrazin test for sugar. To another part a little of the iodo-potassic-iodide solution may be added, to show the presence of starch. The remainder of the fluid may now be distilled, and the residue extracted with alcohol and ether (see chapter on *Urine*); the extract boiled with water and filtered; the filtrate partially evaporated; treated with dilute sulphuric acid, and boiled; saturated with caustic soda; treated again with cupric sulphate, and boiled. The reduction phenomenon will show the presence of dextrin and gums (*Hoppe-Seyler*).[112]

The property of benzoyl chloride to form insoluble compounds with carbohydrates in alkaline solutions may also be utilised as a test.

6. Acids.

(*a.*) **Bile Acids.**—For the detection of bile acids, the distillation residue of the fæces may be tested in the manner already described in connection with the blood (p. 49). If biliary acids be present in great quantity, the application of Pettenkofer's test directly to a watery extract of the fæces (p. 50) will suffice for their recognition. They may also be detected by the addition of a watery solution of furfurol and sulphuric acid (p. 50, and chapter on *Urine*).

(*b.*) **The Volatile Fatty Acids.**—To obtain these bodies from the fæces, the following plan may be adopted:—Dilute the fæcal mass with water, add phosphoric acid, and distil. The distillate contains these acids together with indol, phenol, and scatol. If this distillate be neutralised with carbonate of soda and again distilled, the indol, phenol, and scatol will pass over, and the sodium salts of the fatty acids remain behind. Let these now be evaporated to dryness on the water-bath, the residue extracted with alcohol, and after the evaporation of the latter dissolved in water. The solution may then be tested for fatty acids.

The separation of the different fatty acids may be accomplished by fractional distillation when they are present in abundance. Moreover, they may be partially isolated by precipitation of the sodium salts with æther in alcoholic solutions of varying degrees of concentration (*V. Jaksch*).[113] When there is sufficient material at hand, it is a good plan to convert the acids into their silver or barium salts, and to effect their separation on the principle of their different degrees of solubility in water.

The estimation of the silver, barium, or sodium constituent in the

corresponding salts may be effected in the reactions given below, and the proportion of the respective acids determined accordingly. Of the volatile fatty acids, butyric and acetic acids are those most readily to be found in the fæces.[114] Formic and propionic acids do not appear to have been recognised with absolute certainty, but we shall take account of them here, since it is certain, at any rate, that they occur in another secretion (urine).[115]

(*a.*) **Formic Acid.**—This is a colourless liquid, of a pungent, penetrating odour, which freezes at 0° C., boils at 100° C., and is miscible with alcohol and water.

1. Free formic acid is not precipitated by nitrate of silver, but this reagent will precipitate the alkaline salts of the acid from concentrated solutions. The silver compound blackens quickly in the cold, and when heated reduction takes place.

2. If a solution of perchloride of iron be added to a solution of a neutral salt of formic acid, a blood-red colour appears. This hue disappears on boiling, and a rust-coloured sediment remains.

3. If formic acid or an alkaline salt of the acid be heated with mercuric chloride to 60° or 70° C., a precipitate of subchloride of mercury forms. This reaction is impeded by the presence of free hydrochloric acid or of excess of an alkaline chloride.

(*b.*) **Acetic Acid** is a fluid with an acrid, pungent odour. Its boiling-point is 119°, and it crystallises at 0° C. Heated with ferric chloride, its salts behave like those of formic acid. Nitrate of silver yields a precipitate in neutral solutions of a salt of acetic acid, and this precipitate dissolves in boiling water without being reduced.

When a salt of this acid is heated with sulphuric acid and alcohol, the characteristic odour of acetic æther is obtained.

(*c.*) **Propionic Acid.**—This is an oily fluid. It boils at 117° C. Propionic salts exhibit the same reactions with nitrate of silver as those of formic acid. With ferric chloride they do not yield a red colour.

(*d.*) **Butyric Acid.**—In the pure state butyric acid is an oily fluid, with a goat-like odour, which boils at 137° C. It is miscible in all proportions with alcohol and ether. Its salts, when treated with mineral acids, develop the characteristic goaty smell. Ferric chloride solution does not give a red colour in solutions of such salts, while nitrate of silver forms a crystalline sediment which is insoluble in cold water.

To isolate butyric acid from the isobutyric acid in the fæces, the portion which distils at 158° C. should be treated with carbonate of guanidin (*Brieger*[116]), and the guanidin salt obtained converted by heating into the corresponding guanamin. The base examined under the microscope will then exhibit the characteristic pointed rhomboids of the guanamin of isobutyric acid.

Valerianic, caproic, and others of the higher fatty acids are also present in the fæces. *Weyscheider*[117] asserts that oleic, palmitic, stearic, capric, and caproic acids occur in the discharges of infants.

7. Phenol.—This substance is always a constituent of fæces. When the fatty acids have been converted into their sodium salts in the process described above for the separation of the volatile fatty acids, phenol passes over in the distillate. To isolate it from indol and scatol, the distillate must be rendered alkaline with caustic potash and again distilled. The phenol remains behind, and may be purified by distillation with sulphuric acid.

(1.) If a portion of the distillate now be treated with a solution of ferric chloride, a violet colour will show the presence of phenol.

(2.) The addition of bromine-water to another portion will cause the deposition of a crystalline sediment of tribromophenol.

(3.) Millon's reagent gives a red colour.

8. Indol and Scatol.—Both these bodies occur in the fæces, the latter having been detected there by *Brieger*.[118] To separate them from the phenol present, the distillate in the above process (see *ante*, *Volatile fatty acids*) should be treated with an alkali and again distilled, when indol and scatol will pass over. Indol forms in small colourless scales like those of benzoic acid. It dissolves in boiling water and very readily in alcohol, and is decomposed by strong alkalies.

Scatol, which also crystallises in colourless scales, is much less readily soluble in water, and possesses a disagreeable pungent smell. It is not decomposed by fairly strong alkalies.

To obtain either of these bodies separately, we avail ourselves of the lesser solubility of scatol in water, or of its property of resisting the action of alkalies.

(1.) If nitric acid which contains nitrous acid be added to a solution of indol, a distinct red colour is produced, or a red precipitate if the solution be concentrated.[119]

(2.) Pine-shavings steeped in hydrochloric acid are turned red in an alcoholic solution of indol. The first test, when applied to scatol, does not produce a red coloration, but at most a slight turbidity. The second entirely fails. Jointly they will serve sufficiently for the discrimination of these two substances.[120]

9. Cholesterin, Fats, and Non-Volatile Organic Acids.—Cholesterin, as we have seen, seldom occurs as crystals in the fæces, but, according to *Hoppe-Seyler*, is in one form or the other an invariable constituent of those discharges. For its detection chemically, the residue which remains when the volatile fatty acids and phenol group have passed over in the process of distillation is to be treated with excess of sulphuric acid, and extracted first with alcohol and then with

æther. (1.) The æthereal extract is filtered, the æther removed by distillation, and the residue first digested with carbonate of soda on the water-bath, so as to remove any traces of the volatile fatty acids that may have failed to pass over with the æther, evaporated to dryness, and again extracted with æther. (2.) The alcoholic extract is also filtered, treated with carbonate of soda, the alcohol distilled off, the residue dissolved in water, and finally, as before, extracted with æther. In the alkaline watery residue are contained the biliary acids (*v. supra*), oleic, palmitic, and stearic acids, which, according to *Hoppe*, may be separated by converting them into their barium salts.

Cholesterin and fat are taken up by the æther. The latter is evaporated, the residue treated with alcoholic solution of caustic potash, the alcohol removed by evaporation on the water-bath,[121] and the remaining fluid diluted with water and extracted with æther. The fats remain as soaps in the watery solution, while the cholesterin is dissolved by the æther.

Cholesterin may be recognised by the following tests:—

(1.) A little concentrated sulphuric acid applied to the crystals on a slide will cause them gradually to colour a reddish-yellow round their borders, and finally to grow smaller and disappear.

(2.) When the crystals are dissolved in chloroform and sulphuric acid is added, a blood-red colour forms, presently changing to purple-red. The sulphuric acid at the same time shows a strong green fluorescence.

The soap-solution obtained in the above process is to be rendered acid with dilute sulphuric acid, and the resulting fatty acids removed by filtration. If the filtrate be neutralised with ammonia, it will be found to contain glycerine. The fatty acids may be identified, first by ascertaining their melting-points, and again by converting them into their barium salts, and determining in each case the value of the barium constituent (*Hoppe-Seyler*).[122]

Fats and cholesterin are present in all fæces. Acholic stools present them in relatively large quantities.

10. Colouring Matters.

1. Urobilin.—This is the normal colouring matter of the fæces (p. 126). It can readily be obtained from the stools by treating them with acid alcohol.

Mehu's[123] method for the isolation of urobilin in fæces yields excellent results. A watery extract of the fæces is made, and to this is added sulphuric acid in the proportion of 2 grms. to the litre, and solid ammonium sulphate. The fluid is then filtered, and the precipitate washed with a warm saturated solution of ammonium sulphate, dried on a water-bath, and extracted with a boiling alcoholic solution of ammonia. Urobilin may be shown in the extract so formed

by its characteristic spectrum in acid solutions. This exhibits a well-defined absorption-band between the lines *b* and *F* (*Fraunhofer*) of the solar spectrum (fig. 111).

It should be noted that urobilin may be present even in acholic stools.

2. Blood Colouring Matter.—Pure blood occurs in the stools only after very profuse and rapid hæmorrhage into the intestine. In all other cases where its constituents are found they are greatly altered. Hæmatoidin crystals are rarely seen. Hæmatin is the form in which blood pigment occurs most commonly. This substance may best be recognised by Teichmann's test, or with the spectroscope (pp. 38, 39).

3. Bile Pigment.—The fæces never contain bile pigment in health, but it is found abundantly in the discharges in cases of catarrh of the small intestine. It may be best detected by the application of *Gmelin's* test (nitric acid). On the addition of a little impure nitric acid to a specimen of fæces in which bile pigment is present, the mass changes colour quickly, and the separate drops of the acid are surrounded with rings of green, red, and violet. The appearance of a green ring is very characteristic of bile pigment, and is due to the formation of biliverdin. We have already spoken of the other pigments which may occur in the stools (see p. 125).

11. Intestinal Gases.—These consist of hydrogen, carbonic acid, and volatile carburetted hydrogen (methan).[124] It is not yet definitely determined whether sulphuretted hydrogen is formed in the intestine or not. *Senator* and *Ottavio Stefano*,[125] however, maintain that in certain morbid states this gas is generated in such quantity as to cause symptoms of poisoning. The fact that sulphide of bismuth is formed in the alimentary canal when nitrate of bismuth has been taken (*v. Jaksch*), lends probability to the assumption that sulphuretted hydrogen is also produced there. [See Appendix XV.]

12. Ptomaines.—Putrescin and cadaverin are present in the stools, and may be recognised by the methods already laid down (p. 119). Moreover, the ptomaines known to be produced by pure cultivations of certain pathogenic fungi have recently been obtained directly from the fæces, as by *Pouchet* [126] in cases of cholera. See the methods described at p. 119.

13. Ferments.

Diastase and **invertin** are generally present in the stools of healthy children (*v. Jaksch*).[127] For the detection of diastase see p. 55.

B. **Inorganic Substances.**—The consideration of such inorganic substances as assume a crystalline form has already engaged our attention (p. 155). Chloride of sodium may be detected in the fæces thus:—An extract is made with water, acidulated with nitric acid, filtered, and the filtrate tested with nitrate of silver. A white precipitate

(chloride of silver), soluble in ammonia, will show the presence of the sodium salt.

In *Hoppe-Seyler's* [128] method for the quantitative analysis of inorganic matter in the stools, the substances which are soluble in alcohol are separated from those which are soluble in dilute acetic and hydrochloric acids before the process of incineration is commenced. If this is not done, there is danger that the nuclein, which is nearly always present in fæces, will be decomposed, setting free its phosphoric acid, which may then either remain uncombined or displace other acids from their compounds. The analysis, both quantitative and qualitative, of the incinerated ash is conducted according to methods which are sufficiently familiar.[129]

IV. EXAMINATION OF THE MECONIUM.—The term "meconium" is applied to the substance discharged from the rectum of the child immediately after birth. It is a thick, sticky, viscous fluid, of a greenish-brown colour. When examined by the microscope, meconium exhibits some intestinal epithelium, cells, fatty particles, both fluid and solid,[130] numerous cholesterin crystals, and a variable quantity of well-formed crystals of bilirubin. There are immediately after birth no fungi, and (according to *Escherich* [131]) no spores. After the lapse of twenty-four hours, however, the discharges exhibit a very different character. They now contain abundance of micro-organisms, and Escherich obtained from them by Koch's plate-cultivation methods three distinct microbes.

After the child has taken the breast, the bacteria of the stools, according to the same authority, are represented by two species of micro-organisms. The first consists of thick, curved, rod-like bodies, measuring 1–5 μ in length by 0.3–0.4 μ in thickness. The other is a micro-organism which closely resembles the lactic-acid bacillus of *Hüppe*.[132]

In addition to the above, the meconium contains numerous squamous epithelium cells, derived from the pharynx and œsophagus, or from the anal orifice (*Bizzozero*).[133]

Zweifel and *Hoppe-Seyler*,[134] who have investigated the chemical constitution of the meconium, found it to contain bilirubin, biliverdin, and biliary acids, but no hydro-bilirubin (urobilin). *Wegscheider* [135] found traces of peptone, fats, and soaps, bilirubin, and traces of hydro-bilirubin in infantile stools.

V. CHARACTER OF THE FÆCES IN CERTAIN INTESTINAL AFFECTIONS.

1. Acute Intestinal Catarrh.—In this condition the quantity of the stools is subject to great variety. They are usually fluid, thin, and

slimy, yellowish-brown in colour, and emit a most unpleasant smell. Their reaction is alkaline, except in the case of acute enteritis of children, when it may be acid. Such stools usually contain great quantities of mucus, and there are often visible to the naked eye food remnants in great quantity.

Microscopical examination reveals an abundance of fungi of various descriptions, large quantities of intestinal epithelium, and isolated leucocytes.

2. Chronic Intestinal Catarrh.—In this disease the stools exhibit no very distinctive characters, whether to the naked eye or microscopically.

Nothnagel[136] lays down the following rules for the localisation of chronic idiopathic intestinal catarrh, according to the character of the fæces :—

1. When the large intestine is alone involved, a single discharge takes place within twenty-four hours. Diarrhœa, however, is apt to recur at certain regular intervals.

2. When the small intestine alone is engaged, the motions are also likely to be sluggish.

3. When both the large and small intestines are the seat of catarrh, continuous diarrhœa is apt to ensue.

4. Solid or semi-solid stools containing hyaline particles of mucus, which can be recognised only with the microscope (see p. 127), and devoid of mucus visible to the naked eye, point to implication of the upper part of the large intestine.

5. The presence of bile pigment in the stools, as shown by Gmelin's test, invariably indicates a catarrh of the ileum and jejunum. In such cases also the fæces are usually found to contain epithelium cells and mucus, deeply stained yellow by the bile colouring matter.

In certain forms of chronic catarrh, where the large intestine is especially involved, it sometimes happens that the bodies described at p. 126 are to be found in the stools. Such an affection is then called enteritis tubulosa or membranacea; but it is probable that these manifestations accompany other sufficiently dissimilar morbid states. Our present knowledge of the subject is defective.

3. Ulcerative Enteritis.—The diagnosis of this condition is always attended with difficulty. It is usually (though not always) accompanied with diarrhœa. In a questionable case, the appearance of blood in the stools makes ulceration probable; but we cannot derive any conclusive evidence from either the physical or the chemical character of the fæces to establish the diagnosis. A diligent examination of the discharges, however, may in certain specific forms of ulceration disclose the presence of the pathogenic organisms to whose influence

the process is known to be due. The detection of the tubercle bacillus especially is in this way a fact of the utmost clinical significance (see p. 77).

4. Typhoid Fever.—This disease is usually characterised by abundant foul-smelling discharges of the colour of pea-soup. They contain large quantities of bile pigment, a fact which points to a catarrh of the small intestine, and to which also Nothnagel attributes the peculiarly offensive character of the smell emitted.

The reaction of typhoid stools is in all cases alkaline.

Microscopical examination shows numbers of bile-stained epithelial cells, some leucocytes, abundance of triple phosphate crystals, and a profusion of fungi. Nothnagel's clostridia are especially prominent amongst these. The typhoid bacillus, of course, infests the discharges of this disease; but it cannot be distinguished from the other micro-organisms by a simple microscopical examination. This can be done only by the bacteriological methods indicated at p. 138.

The stools of typhoid in its later stages may be those of intestinal ulceration. Thus, when hæmorrhage results from the extension of typhoid ulcers, the fæces will be blackened, and yield the chemical reactions which denote the presence of a derivative of blood pigment (hæmatin).

5. Dysentery.—The discharges of dysentery are subject to a great variety of character; but there is one respect in which they are constant, for they always contain abundance of mucin, and in the author's experience also some serum-albumin and much pepton.

Under the microscope there are to be seen great quantities of leucocytes, intestinal epithelium, and fungi. Tolerably perfect red blood-corpuscles are occasionally visible. The number of these latter varies within broad limits, but the other microscopical appearances are remarkably uniform.

The grosser properties of dysenteric stools, on the other hand, display notable differences. Founded upon these, *Heubner*[137] distinguishes:—

1. *Mucous and muco-sanguineous discharge.*—A pale yellow, viscous, transparent substance tinged with blood, cohering in masses, with or without admixture of fæces.

2. *Sanguineo-purulent discharge.*—A reddish or yellow fluid, containing flocculent or solid particles as large as a pea or a bean. Such stools may be compared to raw minced meat.

3. *Discharge of pure blood.*—This occurs when a vessel has been opened by the extension of a dysenteric ulcer.

4. *Discharge of pure pus.*—This consists almost exclusively of leucocytes, and belongs to the later stages of dysentery.

5. *Gangrenous stools.*—Such stools are brownish-red or brown-black

in colour, from the presence of altered pigment. They emit a putrid odour. They indicate extensive gangrene of the intestinal mucous membrane.

It was in dysentery that the stools were first noticed to contain those mucous particles which have been compared to frog-spawn (see p. 126), and which Nothnagel afterwards observed in other intestinal diseases. They have no special clinical significance in this disease.

On the whole, it may be said that the naked-eye characters of dysenteric stools are so remarkable that they will ordinarily suffice to establish a diagnosis without the aid of the microscope.

Amœbæ, which have lately been found in such stools, have been credited with a causal relation to the disease (*Hlava* and *Kartulis*).[138] A similar importance is attached by others to a pathogenic fission-fungus (*Klebs*, *Chantemesse*, and *Vidal*).[139] The researches of the last two observers have made it seem probable that the true agent in dysentery is a bacillus which can be cultivated artificially.

6. Cholera.—During an epidemic of cholera, there is usually prevalent a form of diarrhœa which is distinct from that disease, and it is of the utmost importance to possess the means of discriminating between the two. The discharges of the less formidable complaint are not characterised by any special changes; but, in a doubtful case, the investigation in the stools for cholera bacillus (as indicated at p. 133) may be needed to establish the diagnosis.

In a pronounced case of Asiatic cholera, on the other hand, no kind of ambiguity can exist. The discharges are thin, and devoid of smell and colour. They have been aptly termed "*rice-water*" stools. Microscopically they abound in leucocytes and epithelium, and their specific micro-organism, the comma bacillus, may be readily detected. It must, however, be borne in mind that "*rice-water*" *stools are not by themselves pathognomonic of cholera.* They are seen repeatedly in heat-apoplexy and arsenical poisoning; and in such connection, as well as in cholera, they hold a profusion of intestinal epithelium. It follows that the diagnosis of Asiatic cholera will rest on an absolutely secure basis only when the comma bacillus has been found, separated from the stools, and cultivated by the methods with which we are already familiar (p. 135). Chemically, the discharges of cholera contain serum-albumin[140] and much mucin.

7. Hæmorrhagic Stools.—Blood is discharged with the stools in cases of great venous congestion of the intestine, in typhoid, tubercular and dysenteric ulceration of the stomach or intestine, and in round ulcer of the stomach or duodenum. These cases are always attended with symptoms of severe intestinal trouble. The blood is usually profoundly altered (p. 129); but when the hæmorrhage has taken place

in the lower part of the alimentary canal, as the sigmoid flexure or rectum, pure bright blood may be passed.

8. Acholic Stools.—The stools may be deficient in bile in cases of jaundice from obstruction of the biliary ducts, or they may be so in the absence of this condition.

They are characterised by (1) their whitish-grey colour, (2) the abundance of fat which they contain, and (3) a profusion of fat crystals, probably soaps of soda, lime, and magnesia (fig. 72).

Such stools in connection with jaundice imply an obstruction to the flow of bile by blocking of the ducts. When they occur independently of obstruction, the underlying cause is not yet sufficiently understood. Many theories have been framed to account for the phenomenon :—(1.) It may be either that the bile pigment has undergone some change in the intestine which prevents the formation of its metabolic product (urobilin); or (2.) the secretion of bile may be so scanty that there is not enough pigment for the elaboration of urobilin; or (3.) it is possible in such cases that the latter is replaced by certain colourless metabolites of bilirubin (*v. Nencki's* leucohypobilin). The latter view is supported by the fact that considerable quantities of urobilin may be obtained from acholic stools by extraction with acid alcohol (*v. Jaksch, Pel, Le Nobel*).[141] The stools may be devoid of bile in cases of the most varied origin—as in tuberculosis of the intestine, chronic nephritis, and chlorosis—where no trace of jaundice is present. They are commonly so in the fatty discharges which accompany indigestion in children (*Biedert*).[142] It follows, therefore, that we cannot infer the character of the stools from the presence or absence of this symptom. But in all cases where colourless stools concur with jaundice, the cause is to be found, as has already been said, in obstruction of the biliary passages.

CHAPTER VII.

EXAMINATION OF THE URINE.

The urine is the secretion of the kidneys.* A sufficient and exhaustive knowledge of the characters of this secretion is a point of the utmost consequence to the physician, since the changes which it undergoes are the expression of numerous morbid processes, and their intelligent interpretation affords the surest aid to diagnosis.

I. NAKED-EYE INSPECTION OF THE URINE.

1. Quantity.—The quantity of urine secreted in health varies within broad limits, and depends at any time upon the relation subsisting between the imbibition and abstraction of fluids in the system. It follows that an error as to excess or deficiency can be considered morbid only when very marked. In general terms it may be said that a healthy able-bodied man will pass 1500 to 2000 cc. of urine in twenty-four hours.

Under pathological conditions, however, the healthy standard may be widely departed from in either direction.

In order to estimate the quantity of urine secreted, that which is passed within twenty-four hours should be collected; and it is well to date the period from eight o'clock of one morning to eight o'clock of the next. When only half this interval is taken—and especially when the estimation of urea is in question—it is important that the bladder should be previously emptied. The patient should be admonished to make water before going to stool, and even then a certain allowance must be made for urine passed in the act of defæcation. Should the patient be the subject of incontinence, it is very difficult to form an accurate estimate of the quantity of urine secreted; and this can only be done by passing the catheter as often as possible—hourly, if it may be—so as to minimise the escape of fluid. In paralysis of the bladder, while sensation remains unimpaired, the escape of urine may be pre-

* We shall confine ourselves here to a description of the simpler processes, and such as are likely to be applied clinically. For more exhaustive information the reader is referred to the text-books of urinary chemistry (see references 1 and 2).

vented by the use of a permanent receptacle. The urine saved should, in any case, be placed in a vessel of two litres capacity, and provided with a graduated scale showing its capacity in cubic centimetres. Its quantity may be most accurately estimated by weighing it.*

A diminution of the quantity of the urine (oliguria) occurs in febrile conditions, in disturbances of the circulation of all kinds, and especially of the capillary circulation, in acute, and in some forms of chronic, nephritis. The urine is increased in quantity in diabetes mellitus, diabetes insipidus, contracted kidney, amyloid degeneration of the kidney, and usually in convalescence after acute diseases. Under the latter heading the increase is most pronounced in the non-febrile period of relapsing fever, at the termination of an attack of acute nephritis —whether cure be impending or the acute is passing into the chronic form of the disease—and in the restoration of the balance in the capillary circulation, as where compensatory changes take place in heart-disease, &c. Finally, the renal secretion is promoted by certain drugs, as the salts of potash, salicylates, digitalis, and calomel.

A notable diminution of the urinary secretion (anuria) is an accompaniment of uræmia and of all diseases that are attended with the abstraction of water from the system: such are severe acute anæmia, gastric and intestinal catarrh, cholera, and dysentery. The transitory suppression of urine which sometimes occurs in healthy persons after profuse perspiration, and lasts only a few hours, is altogether physiological. A simple increase or diminution of urine will not by itself establish the nature of a disease, but it is always an important factor in diagnosis, and we shall see by and by that the consideration of this point will enable us especially to discriminate between certain forms of kidney-affection.

2. Density of the Urine (*Specific Gravity*).—The density of the urine varies greatly in health, and is for the most part in inverse ratio to its quantity. If we assume the latter to be on an average 1500–2000 cc., then the sp. gr. of healthy urine may be stated at 1.020 to 1.017. It may be estimated most accurately by means of the pycnometer (see text-books), but for clinical and practical purposes an instrument constructed on the principle of the hydrometer will serve well. Such an instrument, when employed for testing urine, is called a urinometer. It is well to have two in use, one for taking the specific gravity when this lies between 1.000–1.025, the other where it is as high as 1.025–1.050.

A serviceable urinometer should be so made that the degrees on the index-scale shall be separated by a sufficient interval, not less than 1 mm.; and when very accurate results are aimed at, it should be

* See the text-books referred to, ref. 2.

graduated in tenths. Moreover, it should be furnished with a thermometer, also graduated in tenths of a degree, and recording temperatures between 0° and 30° C.

A new urinometer should be tested in distilled water. If accurate, it will sink to the mark 1.000, where the scale is graduated so low as this.

The specific gravity is tested in the following manner:—The urine is poured into a cylindrical glass vessel of suitable width. Should froth form, it must be removed either with filter-paper or by filling the vessel to the brim, when it may be blown off. The urinometer is then placed in the urine, and care is taken that it is not anywhere in contact with the sides of the vessel. When it is quite stationary in the liquid, the specific gravity is read off, the observer bringing his eye on a level with the concave surface of the liquid. The mark on the scale which corresponds to the lowest point of this concavity will indicate the specific gravity of the fluid.

Greater accuracy may be attained by testing the urine at some particular temperature, for which the urinometer has been constructed.

An abnormal specific gravity of the urine is a fact of great importance in disease. It affords an approximate estimate of the quantity of solids excreted by the kidneys, and consequently of the energy of the metabolic processes within the system. It may be stated as a general rule, that when the quantity of the urine is diminished in disease, its specific gravity is raised. A considerable departure from this rule implies one of two things:—Either tissue changes are notably suspended, and their products, urea, uric acid, &c., formed in smaller quantities; or these processes remaining active, such products fail to be removed by the kidneys. To the first of these causes is to be assigned that rapid decline in the density of the urine which sometimes precedes a fatal termination in acute fevers. Of still more serious import is a sudden fall in the specific gravity in nephritis, unattended with any alteration in the quantity of urine passed. The phenomenon in this instance points to the failure of the diseased kidneys to separate the urea and salts elaborated within the system. The author has had many opportunities of observing that such a fall in the specific gravity is apt to precede—usually by several days—the oliguria and suppression which herald an attack of uræmia; and it often affords a valuable warning of what is impending at a time when all other symptoms are wanting. Moreover, the symptoms of uræmia may develop whilst the urine remains but little diminished in quantity, and in such cases we shall always find that its specific gravity is greatly lessened.

3. The Colour of the Urine.—The normal urinary pigments have not yet been separated. *C. Vierordt*[3] concludes from spectroscopic

appearances that they are several in number. Up to the present, however, but two chromogens have been determined in the urine, viz., indican (sulphate of indoxyl; see *Indicanuria*), and the chromogen of urobilin.

The colour of healthy urine depends upon its degree of concentration, being darker as this is more pronounced.

The same statement holds in general for disease, but with notable exceptions; for there are some affections in which a high colour and an abundant flow coincide, and others in which the urine is at once pale and scanty.

In some diseases, and especially in fever, additional colouring matters are secreted. The nature of some of these is a matter of speculation (uroërythrin, urochrome).

At some period in the course of a disease, the urine may undergo a change of colour from the admixture of blood. When the latter is present in small quantity, the secretion may be flesh-water-coloured. When blood is more plentifully effused, it may be a bright ruby-red (see *Hæmaturia*).

Bile pigment imparts a brownish-yellow or green tint. Its presence may usually be detected by shaking up the urine, when a yellow foam will form upon it. It must, however, be borne in mind that urine which contains much *urobilin* will yield a similar foam (*Leo Liebermann*[4]); in the latter case the secretion is always of a dark brown-red (see *Urobilinuria*). An excess of indoxyl sulphates will cause the urine to assume a dark-brown tint, and the yellow foam will not form upon it (see *Indicanuria*). The brown tint in such cases is due, not to the indoxyl salts, which are colourless, but to some other substance which is present with them.

Certain drugs, too, will affect the colour of the urine. Rhubarb and senna cause it to become brown or blood-red; a black colour is developed when carbolic acid is taken into the system, especially if the urine be allowed to stand for some time, and the same appearance follows the exhibition of naphthalin, hydrochinon, resorcin and pyrocatechin. The blackening of the urine by carbolic acid is ascribed by *Baumann* and *Preusse*[5] to the formation of the oxidation-products of hydrochinon. The use of quinine, kairin, antipyrin, and thallin also colours the urine more or less deeply.

It may be stated in general that the urine is darkly coloured in fevers, and in congestion of the kidneys due to heart-disease, emphysema, &c. It is, on the other hand, deficient in colouring matter in diabetes mellitus and insipidus, chronic nephritis, urina spastica, and all kinds of anæmia. In cancer, especially when it implicates the intestinal canal, the urine is apt to be dark and pigmented. In such cases an excess of indican may generally be determined.

Vogel has endeavoured to construct a standard scale for the estimation of colour in the urine. [See Appendix XVI.]

4. The Reaction of the Urine.—Healthy human urine is ordinarily acid. The reaction is due not to free acid, but to the acid salts (phosphates and urates) which it contains.

It is, however, subject to considerable variations in this respect. *Quincke*[6] has determined that the acidity is in general less in the forenoon than at any other time, and the urine of healthy persons may even exhibit an alkaline reaction then.[7]

The reaction of the urine is also modified by diet. It may be alkaline after an ample meal, or the ingestion of alkalies and of substances such as the salts of acetic, tartaric, and citric acids, which are converted into carbonates in the system. The administration of acids, on the other hand, renders the acidity of the urine more marked.

Healthy urine becomes alkaline when allowed to stand for some time, in consequence of the action of a micro-organism, the micrococcus ureæ (see p. 189), which decomposes the contained urea into carbonate of ammonia. It occasionally happens that the same urine will turn red litmus paper blue, and blue litmus red, *i.e.*, it is amphoteric. This depends upon the presence in it of acid or neutral phosphates (*Huppert*).[8]

In different morbid states the freshly passed urine may be either alkaline or acid; but its reaction in disease is valuable as a symptom only when the causes which are known to influence it in health can be excluded. A fact of the greatest consequence in this connection is the alkalinity due to ammoniacal fermentation, and this can usually be ascertained directly by the sense of smell. The urine is, as a rule, acid in febrile conditions, diabetes, and leukæmia; in scurvy, too, it is apt to be intensely acid. On the other hand, it is alkaline in simple and pernicious anæmia and chlorosis. According to *Bence Jones*, the alkalinity in these cases depends upon the deficient formation of acid in the stomach. In chlorosis the point is of interest to the physician, insomuch that whilst the reaction of the urine continues to be alkaline, it may be inferred that the morbid process on which it depends is still going on. Ammoniacal urine implies ammoniacal fermentation within the bladder. This may be due to the use of an unclean catheter, and commonly arises in the course of cystitis.

The reaction of the urine is best tested with red and blue litmus paper. The comparative estimation of acidity may be effected by *Huppert's* method.[9] [See Appendix XVII.]

II. MICROSCOPICAL EXAMINATION OF THE URINE.—Healthy urine is generally quite clear when first passed. On standing, if not decomposed by the rapid development of fungi, it deposits a filmy

cloud. Microscopically, this deposit is seen to consist of a few crystals of various kinds, some white blood-corpuscles, and epithelial débris. But the appearance of freshly passed urine may differ greatly from this, even in health. The concentrated urine passed in the morning sometimes deposits an abundant sediment of urates altogether independently of disease.

The examination of morbid urine affords information of the utmost consequence in diagnosis. Such urine may either be turbid when passed, or it may deposit a variable quantity of sediment when allowed to stand for some time; and when this sediment is looked at through the microscope, it is seen to hold certain substances of very variable character. For convenience of description we shall divide these substances into two classes, the *Organised* and the *Unorganised* urinary deposits.

The microscopical examination of the urinary sediment may be conducted in the following manner:—The urine is allowed to settle, the clear supernatant fluid poured off, and some of the sediment placed in a conical glass, when it is again allowed to stand for a while. A little is then removed with a pipette and placed on a slide for examination. If the sediment should be scanty and require a long time—say twenty-four hours—to deposit, it should be set apart in a cool place, so as to check the excessive development of fungi and fermentative processes, which might alter the character of the specimen. It is well also to add some indifferent antiseptic substance to the urine, as thymol, hydriodic acid, or oil of turpentine. An admirable admixture is that of *Salkowski*[10]: 20 to 30 cc. of a fluid containing 5 to 7.5 cc. of chloroform in a litre of water. Carbolic acid should not be employed, because it will cause a precipitate with any albumin which may be present.

1. Cellular Constituents (Organised Sediment) of the Urine.

1. Red Blood-Corpuscles. — Red blood-corpuscles occur in variable quantity as a morbid constituent of the urine. They may be so few in number as not to affect the colour of the fluid, and to be discernible only with the microscope; or they may be present in such abundance as to form a layer several centimetres deep at the bottom of the vessel, and when intimately mixed with the urine impart to it a deep red colour.

The condition in which they are found is not less liable to vary than their number. They may retain their proper form, or they may appear as pale yellowish rings (phantom corpuscles of *Traube*). (See fig. 77.) Important inferences as to their origin are to be drawn from the number and character of the red blood-cells in the urine. Such cells may be derived from the urethra, the bladder, the ureters, the renal pelvis, or the kidneys themselves.

When they are intimately mixed with the urine, in such a way that

though present in large quantity and deeply tinging the fluid, they do *not* form a sediment after many hours' standing, it may be inferred that the hæmorrhage took place in the substance of the kidney or in the renal pelvis or ureters. If, under these circumstances, they are seen with the microscope to be profoundly altered, having lost their colouring matter and presenting the appearance of pale yellow rings, the further conclusion results that the blood was effused in the kidney itself, and the symptom points to acute nephritis or to a fresh exacerbation in the course of chronic nephritis. When the blood-cells appear as comparatively few, attenuated, and washed-out rings, they may have originated in congestion of the kidneys or in miliary tuberculosis of those organs, and the diagnosis of these conditions may be established on this ground—in conjunction, of course, with other symptoms.

It is more difficult to decide from the character of the blood passed whether the lesion has taken place in the renal pelvis or the ureters. To arrive at a just conclusion on this point, a careful examination must be made for the other occasional organic constituents of the urine, as epithelium, casts, &c., and the diagnosis will rest in a large measure upon their character (see pp. 177 and 180).

When blood is present in considerable quantity without being intimately blended with the fluid, it is derived in the majority of cases from the bladder. Intermittent hæmaturia, attended with severe pain, is caused by calculi or tumours in that organ.

2. **Leucocytes.**—The urine contains isolated leucocytes in health. It is only when they occur in greatly increased quantity, or in conjunction with other cellular elements of a pathological character (casts), that their presence attains any serious import. They are usually unaltered in form, but sometimes, and especially in alkaline urine, they swell up, become glossy and homogeneous, and their nuclei disappear, but can again be made visible with acetic acid. Occasionally they enclose much fatty matter, and this chiefly when they are derived, not from the urinary passages themselves, but from the bursting into them of a slowly-formed abscess of some neighbouring organ, as the rectum or prostate.

Leucocytes are occasionally seen to present protoplasmic processes. This happens when the secretion possesses a feebly alkaline reaction.

The leucocytes of the urine may be derived from the substance or the pelvis of the kidney, the ureters, the bladder, the urethra, or, as already mentioned, from the rupture of an abscess into some part of the urinary passages.

These bodies may form a compact layer of sediment several centimetres deep in purulent catarrh of the bladder, and a similar deposit of pus has been found in the urine in cases of acute infectious urethritis

(*v. Jaksch*). Such pus is thick and viscid, and the constituent leucocytes are usually much altered in character (*vide supra*).

Pus cells occur in the urine in considerable quantity in inflammation of the ureters and in pyelitis, but in this connection they are never so abundant as in cystitis, and they are commonly deposited in the form of a flocculent sediment of a slimy translucent appearance, which, when looked at through the microscope, is seen to consist of a more or less dense aggregation of leucocytes. The distinction as to the nature of the sediment is not, however, altogether characteristic, nor is it in either case invariable. With this qualification, it may be recommended as a point worth attending to in the differential diagnosis of cystitis and inflammation of the pelvis and ureters.

In renal disease there are but few leucocytes to be found in the urinary sediment, unless in the rare cases where a renal abscess discharges directly into the large tubules or the pelvis of the kidney.

Care must be taken in the case of women to ascertain whether pus found in the urine may not have been derived from the vaginal secretion. In blennorrhœa a considerable quantity of pus may find its way from this source into the urine.

When pus in great quantity appears suddenly in the urine (pyuria), it points to the opening of an abscess into the urinary passages, but cases are on record (*v. Jaksch*) where no such causes could be assigned, and these constitute a rare but remarkable exception to the principle laid down. They depended, doubtless, upon unusual conditions favouring diapedesis.

Leucocytes can generally be recognised by the microscope; but should any doubt arise as to their nature in a particular case, it may be at once resolved by the addition of a little iodo-potassic-iodide solution. With this reagent the leucocytes stain a deep mahogany-brown (glycogenic reaction), whilst the forms of epithelium, with which they are occasionally blended, and which may be confounded with them, assume a light yellow colour.

3. Epithelium.—The slight cloud which ordinarily forms in healthy urine contains a number of epithelial cells. These are for the most part of the squamous variety; but amongst them are also some smaller forms, which are derived chiefly from the mucous surface of the renal pelvis and ureters, and very rarely from the substance of the kidneys.

In addition to these, there are to be seen in every specimen of urine a considerable number of uninuclear polygonal cells, and similar round cells, which are their earlier form. These belong to the meatus and prepuce, and in women to the vagina. Their presence in comparatively small numbers has no special significance; but when they occur in excess, they indicate a catarrh or catarrhal irritation of the parts

from which they are derived. A form of epithelium, consisting of oblong cylindrical cells, diminishing in size towards their attached extremity, and with well-defined borders, comes from the surface of the male urethra (*Bizzozero*).

It is very difficult to distinguish between the epithelial cells which are derived respectively from the bladder, ureters, and renal pelvis. *Bizzozero*[11] maintains that the cellular type is the same for all these parts, and *Eichhorst*[12] agrees with him.

It follows that a particular affection in one of these situations can hardly be localised by the character of the urinary epithelium. The cells in each case have this in common, that they are smaller than those already described. Those that come from the superficial layers of the mucous membrane are polygonal or elliptical in shape. They usually have a single large nucleus, and their protoplasm is apt to be

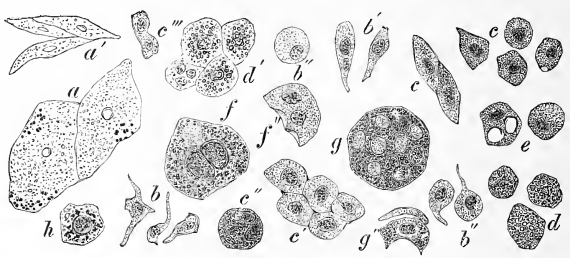


FIG. 74.—Epithelium from the Urinary Sediment.

a, *a'*. Squamous epithelium from the urinary sediment.
b, *b'*, *b''*. Epithelium from the bladder.
c, *c'*, *c''* *c'''*. Epithelium from the kidneys.
d, *d'*. Fatty epithelium from the kidneys.
e–h. Epithelium from the bladder.

very granular. The cells which are derived from the middle and deeper layers are more oval in shape, often irregular and conical, and furnished with one or two long protoplasmic processes (fig. 74, *b*, *b'*, *b''*). They also have a single nucleus of large size, and their substance is clearly granular. Like *Bizzozero* and *Eichhorst*, *v. Jaksch* has been unable to find any morphological distinction amongst these cells, by which their origin in the bladder, ureter, or pelvis can be known; nevertheless, he is of opinion that certain inferences may be drawn from their number. Given a disease of one of these parts, it may be assumed to involve the ureter alone when the epithelial forms which we have been considering are very few. When in greater quantity and superimposed upon one another like the tiles of a roof, they probably come from the pelvis; and when in very great abundance, they indicate cystitis. These points should not be strongly insisted upon;

but taken in conjunction with other symptoms, they should carry some weight in a differential diagnosis.

The general pathological condition to which the presence of these cells in excess is, in any case, to be ascribed, is that of irritation or inflammation of the mucous surface of the bladder, ureter, or pelvis of the kidney. If the reader will refer to what has been said of the leucocytes in the urine in connection with these diseases, he will see that the character of the sediment in the two particulars taken together, and read in conjunction with the clinical symptoms, will suffice very accurately to discriminate cystitis from inflammation of the pelvis or ureter.

Much importance attaches to the appearance in the urinary sediment of epithelial cells derived from the mucous lining of the tubules of the kidney. Under normal conditions they are distinguished by their smaller size from the forms just considered, or at any rate from such as belong to the middle and deeper layers of the urinary mucous membrane. They are polyhedral in shape, and finely granular, with comparatively large oval nuclei and nucleoli. They occur separately or cohering in groups (fig. 74, *c*, *c'*, *c''*, *c'''*); and in the latter case may display the cylindrical arrangement (Epithelial casts, fig. 76). They are often to be seen, singly or several together, on the surface of the casts to be described later (fig. 85, *c*).

The cells of kidney epithelium exhibit remarkable deviations from the normal type. They are sometimes hard, tough, and glossy, like the obsolete cells of the intestine described by *Nothnagel* (p. 130). They occasionally contain fatty globules in greater or less profusion (fig. 74, *d*, *d'*), and again cells are seen overlying the surface of casts (fig. 84, *a*), which may conform in shape to the type described above, but consist entirely of fatty matter (see also fig. 84, *c*).

In the convalescent stage of acute nephritis (of scarlatina and erysipelas), small round cells with an eccentric resting nucleus are frequently to be seen, and these are doubtless the young kidney-cells formed in the process of repair within the tubules (*v. Jaksch*).

The detection and the character of the kidney epithelium in the urine is a point of the highest import in diagnosis. Its presence is always a sign of renal disease, and usually of inflammation. In addition to this, and where the coincident symptoms indicate a nephritis, the character of the epithelium will enable us to form a probable opinion as to whether the inflammatory process is accompanied with degeneration of the kidney substance. Thus where the cells are found loaded with fat, the autopsy will most likely disclose a fatty condition of the renal tissues; and the detection of the obsolete cells described above points with some degree of certainty to an amyloid degeneration of the organ.

It is needless to add that a diagnosis can never be based upon such appearances alone, but must in all cases be controlled by the clinical symptoms and the other manifestations of the urine.

4. Casts.—The subject of urinary casts, which will now engage our attention, is one of the highest interest. Casts were first seen in the urine by *Vigla*, *Quevenne*, and *Rayer*,[13] in France, and almost simultaneously in Germany by *Simon* and *Nasse*.[14] *Henle*,[15] and subsequently *Rovida*,[16] have contributed the most ample information concerning them. These bodies are subject to very great variety as to their number when present, their form, and the import which attends their manifestation. It must be premised that they have been found in urine which is entirely free from albumin, and even from every other morbid product. Thus *Nothnagel*[17] has seen them in the urine of patients with jaundice, which at the same time contained no albumin; *Burkart* and *Fischl*[18] likewise observed them, in the absence of albumin, in cases of severe inflammatory affections of the stomach and intestine. Hence it follows that the presence of casts in the urine does not by itself imply disease.

Urinary casts may be conveniently divided into two chief classes, viz., *Unorganised* and *Organised*.

(*a.*) *Unorganised Casts.*—These are formed of crystals. They are pathologically of little consequence. Those that have been described consisted of urates (fig. 75) and hæmatoidin; and as yet they have been found only in infants, and in cases of gout and renal congestion. If healthy urine be concentrated in vacuum at a low temperature, 37°–39° C., casts may be observed which consist of acid urate of soda (*Leube*).[19]

Perhaps we ought to include under this heading some of those bodies which are at present classed together under the general name of "detritus" casts.

(*b.*) *Organised Casts* consist of cellular elements, or the products of their transformation.

They may be subdivided into three groups: (1.) Those which consist of cells (red blood-corpuscles, leucocytes, and epithelial cells). (2.) Those which consist of the products of cellular change. (3.) Those so-called hyaline casts, whose origin is still a subject of dispute, but which are sufficiently distinguished from the others, both clinically and morphologically.

(1.) The first group includes:—

(*a.*) Casts formed of red blood-corpuscles (fig. 77).
(*b.*) Casts formed of leucocytes (fig. 78).
(*c.*) Epithelial casts (figs. 76 *a*, *b*, and 79 *a*, *b*).
(*d.*) Casts consisting of colonies of bacteria.

(2.) Under the second group we distinguish :—

(*a.*) Granular, (*b.*) waxy, and (*c.*) fatty casts.

(3.) The group of hyaline casts may be subdivided according as its members are simply hyaline, or, in addition, coated with certain substances, amongst which kidney epithelium, red and white blood-corpuscles, bacteria, and various forms of crystals may be enumerated.

The cylindroids of *Thomas* should perhaps be included in this group.

The number of casts occurring in a specimen of urine, and the length and breadth of each, is subject to very great variety. The latter fact is sufficiently seen in the accompanying figures.

1. Perfectly formed cellular casts appear in the urine only under such circumstances as cause the renal tubules to become crowded with red or white blood-corpuscles, or bring about a separation of the renal epithelium in the entire circumference of a tubule. When this occurs,

FIG. 75.—Cast formed of Urates.

the casts formed are forced onward by the flow of the fluid secreted behind them, and are ultimately discharged from the bladder.

Fig. 79 (*a* and *b*) shows a rare specimen of casts, consisting of renal epithelium and leucocytes from the urine in a case of nephritis with oliguria and uræmia.

The clinical significance of these casts is very great. They always imply an affection of the kidney, and their presence alone suffices to establish the existence of acute nephritis, or the supervention of a fresh paroxysm in that disease. Figs. 76, 77, and 78 represent specimens of cellular casts formed in different proportions of the several cellular elements which enter into the formation of these bodies.

Casts are sometimes found which consist almost entirely of masses of micrococci, but these have quite a different significance. Fig. 88, *d*, represents such a one. Morphologically, they bear a close resemblance to the granular casts presently to be described, but are distinguished from them by their resistance to powerful reagents, such as caustic potash and nitric acid. They may also be known by their opacity and

the grey colour which they display, as well as by the remarkable uniformity of their substance (*Martini*).[20]

Casts of micrococci in the urine are a matter of very grave signifi-

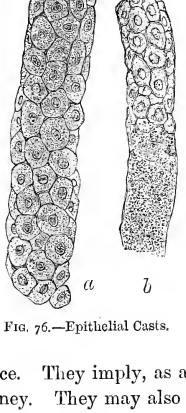


FIG. 76.—Epithelial Casts.

FIG. 77.—Blood Cast.

FIG. 78.—Leucocyte Cast.

cance. They imply, as a rule, the existence of septic embolism of the kidney. They may also arise from the extension upwards of a septic pyelitis (pyelo-nephritis).

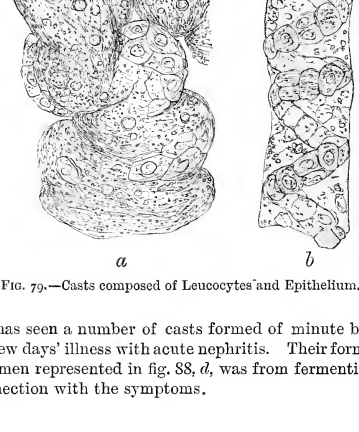
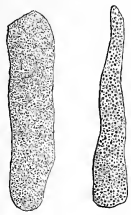


FIG. 79.—Casts composed of Leucocytes and Epithelium.

The author[21] has seen a number of casts formed of minute bacilli in the urine of a boy after a few days' illness with acute nephritis. Their formation was transitory. The specimen represented in fig. 88, *d*, was from fermenting diabetic urine, and had no connection with the symptoms.

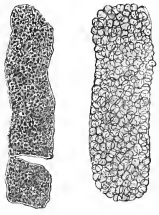
2. The members of the second group, as we have seen, are granular, waxy, and fatty casts.

(*a*.) **Granular Casts** vary much in dimensions. They are most fre-



a *b*

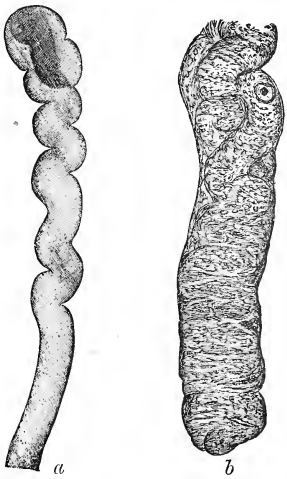
FIG. 80.—Granular Casts.



a *b*

FIG. 81.—Granular Casts.

quently seen in a fragmentary state, but are occasionally of perfect form. Their borders, which are usually well defined, are often sinuous in casts of some length (fig. 82, *a* and *b*). In the latter case, too, they are somewhat concave at the extremities; but when very short and



a *b*

FIG. 82.—Granular Cast.

broken, they present a zigzag ending. Their constitution is very variable. Sometimes they consist of fine particles, which can be distinguished only by a high power of the microscope (fig. 80, *a*), as Zeiss'

objective F. In other cases they are coarsely granular, and the constituent particles can be readily made out with Hartnack IV. (fig. 81, *b*). In colour, too, they manifest considerable differences. They may be of all shades, from pale yellow to a reddish-brown. They are occasionally coated with leucocytes, fatty globules, and needles of fatty crystals (figs. 82, *b*, and 84 *a*, *b*). These distinctions of character are not known to correspond to special derangements of the kidney.

Granular casts sometimes exhibit a transition form of epithelium (fig. 76, *b*), and it is probable that they usually originate in the de-

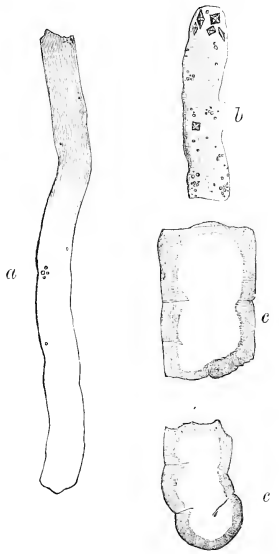


FIG. 83.

a. Waxy cast with urates on its surface; *b*. with crystals of oxalate of lime; *c*. Fragments of waxy casts.

generation of the blood- and epithelial casts already described. This theory was first stated by *Rindfleisch* and *Langhans*.[22]

The presence of granular casts in the urine indicates an inflammatory condition of the kidneys. They have been found (*v. Jaksch*) as an exceptional constituent of the secretion in cyanotic induration of the kidney, and especially when the latter condition was associated with nephritis (secondary nephritis). These bodies may be regarded almost equally with cellular (epithelial) casts as a reliable indication of nephritis.

(*b.*) **Waxy Casts.**—These casts attain a greater length than the others, and when comparatively perfect they may be seen to be segmented like a tapeworm. They also occur in shorter fragments of relatively great breadth. Under the microscope they are homogeneous and refractive, and often bear upon their surface fatty globules, separately or in confluent masses, white and red blood-corpuscles, fungi, and crystals of various kinds.

Their constitution has not been definitely ascertained. It would appear to be very complex, and that such casts result alike from the breaking down of epithelium and from the exudation of occasional products (fibrin, amyloid material) into the renal tubules. Their number also is very inconstant.[23]

They are not characteristic of any special disease. They are found in acute and chronic nephritis, in contracted granular and in amyloid kidneys. They frequently exhibit the amyloid reaction with methyl-

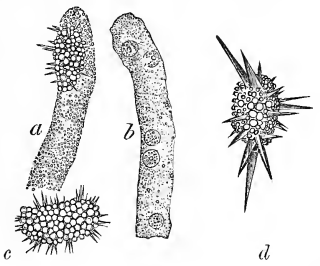


FIG. 84.

a. Granular cast beset with fatty globules and fatty crystals; *b*. Granular casts beset with leucocytes; *c*, *d*. Fatty globule casts.

violet and iodo-potassic-iodide solution; and this in the absence of amyloid degeneration of the kidney. Moreover, the reaction is not obtained in some cases where this condition exists, and consequently no inference can be drawn from its manifestation.

(*c.*) **Fatty Casts.**—Fatty globules are found upon the surface of granular casts (fig. 84, *a*); but they also form by themselves short, powerfully refracting cylinders, which often are beset all over with needles of fatty crystals (fig. 84, *c*, *d*).

These casts and fatty crystals were first pointed out by *Knoll*. They are most commonly associated with subacute and chronic inflammations of the kidney of protracted course, with a tendency to fatty degeneration of the renal tissues (*v. Jaksch*). Consequently their detection affects the prognosis unfavourably.

Post-mortem examination has shown that they form most frequently

in cases of large white kidney. In some instances where they were present, however, the organ was found to be more or less contracted; but when this was so, it was invariably far advanced in fatty degeneration. The crystals which beset fatty casts are not formed exclusively of fat, but probably to some extent of lime and magnesia salts of the higher fatty acids and allied compounds, for they are *not* all soluble in æther. They have their origin doubtless in fatty degeneration of the renal epithelium.

3. **Hyaline Casts.**—Hyaline casts are for the most part pale and delicate bodies, of varying length and thickness, and more or less abundant in different specimens. They cannot, as a rule, be detected without being previously stained. Their pathological significance differs greatly according as they are coated with certain substances or not.

In some diseases, whilst the urine contains no albumin, very pale hyaline casts are found in small numbers in the sediment. *Nothnagel*

a *b* *c*

FIG. 85.

a. Hyaline casts; *b*. Hyaline cast coated with leucocytes; *c*. Beset with renal epithelium.

has met with such in albumin-free urine in cases of jaundice, and *Henle* in others where the kidneys were quite healthy. Their presence, therefore, has no necessary connection with renal disease. The author has repeatedly been able to recognise hyaline casts in the urine in affections whose subsequent course altogether excluded the possibility of diseased kidney; and *Huppert*[24] has shown that the urine voided after an epileptic paroxysm may contain both albumin and hyaline casts in the absence of any kind of inflammation in that organ. According to *Leube*,[25] hyaline casts are seldom met with in urine free from albumin. Nothing more need be said to enforce the precept that the existence of renal disease, or at all events of nephritis, must not be hastily concluded from the mere presence of hyaline casts.

But when these casts bear a coating of other substances upon their surface the case is altogether different. In nephritis we sometimes

find in conjunction with other casts cylinders of hyaline substance which are covered with epithelium (fig. 85, *c*), unaltered or loaded with fat, leucocytes (fig. 85, *b*), and red blood-corpuscles.

In severe cases of jaundice depending upon disease of the liver, as in secondary carcinoma of that organ complicated with nephritis, colourless hyaline casts are formed in the urine, and overlying their substance are golden-yellow cells of kidney epithelium, which colour red, changing to blue in presence of nitric acid.

Urates are seen deposited upon such casts in cases of congested kidney; and other bodies, as oxalate of lime and bacteria, may similarly occupy their surface.

This is perhaps the place where mention should be made of the so-called cylindroids. These are long, ribbon-like bodies, resembling casts, which were first discovered by *Thomas*[26] in the urine of scarlet fever. They occur also in nephritis cystitis and renal congestion, and *Bizzozero* has found them in healthy urine. They are not, therefore, characteristic of kidney disease. They are observed most commonly in the urine of children, which may or may not also exhibit albumin, in the absence of other symptoms. *Pollak* and *Török*[27] have noted these occurring together with abnormal excretion of urates.

With regard to the mode of origin of hyaline casts and cylindroids, *Rovida*[28] suggests that they are the products of secretion by the epithelium lining the urinary tubules, and his view is borne out by the experiments of *Pollak* and *Török*. In this way it is possible to account for their manifestation in the absence of severe renal affections. At the same time it must be borne in mind that the experiments which *Ribbert*[29] made some years ago upon animals point to the conclusion that hyaline casts may result directly from the exudation of albumin within the tubules.

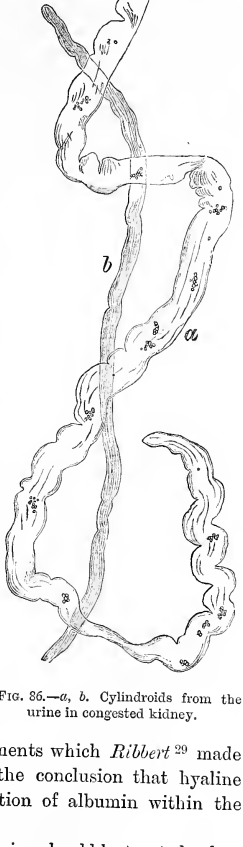


FIG. 86.—*a*, *b*. Cylindroids from the urine in congested kidney.

Method of Examination for Casts.—The urine should be treated, when necessary, with antiseptic agents (see p. 175). It should be covered and

allowed to stand for several hours, and some of the sediment which has then fallen should be removed with a pipette for examination on a slide.

The casts belonging to the first two classes can usually be recognised without special preparation. Simple hyaline casts may need to be stained to render them visible, and this may be done best in their case by the use of a drop of dilute solution of iodine and iodide of potassium. Other staining fluids may be used for colouring the different varieties of casts. These are picro-carmine, gentian-violet, eosin, acid hæmatoxylin solution, safranin, Bismarck-brown, and methylene-blue (*Knoll*). It should be mentioned, however, that the staining properties of casts vary greatly, and that some which are morphologically similar will behave very differently in solutions of the substances enumerated here.

For the investigation of these staining properties the sediment should be washed with the normal saline (·75 per cent. NaCl) solution (*Knoll*[30]), and care should be taken that the dyes employed are sufficiently diluted.

Chemical Constitution of Renal Casts.—Rovida is still the classical authority upon this subject. According to him, the characteristic property of hyaline casts is their solubility in weak mineral acids. This property they possess in common with cylindroids.

Waxy casts in their behaviour with chemical agents resemble albuminates, but they also exhibit reactions which sufficiently distinguish them from the latter substances. It would appear in general that the substance of which casts are formed is not a proteid, but some derivative of one. In this conclusion Rovida had been long anticipated by *L. Mayer*.[31] Knoll has also satisfied himself that the substance of which renal casts are composed is not identical with any of the proteids with which we are familiar, as acid albumin, albumin, albuminate, albumose, globulin, fibrin, mucin, or peptone.

5. Spermatozoa.—These are pear-shaped bodies, about 50 μ in length. Of this, the head occupies 4 to 5 μ, and the remainder is a long tail which tapers towards the extremity (see fig. 124).

Spermatozoa are found in the urine of men after coitus, involuntary emissions, *e.g.*, of epileptic paroxysms (*Huppert*), and masturbation. They may also occur in the urine of women when passed directly after connection (see Chapter IX.).

6. Fragments of Tumours.—It rarely happens that fragments of a tumour are found in the urine. The author has never yet by their aid been able to diagnose a growth in the kidney. On the other hand, a carcinoma of the bladder, or a tumour of some other organ, as the vagina or rectum, which has burst into the bladder, may betray its character by imparting its constituents to the urine. Thus a pigmented tumour may be known by the detection of melanotic particles in the sediment; but in other cases, as where cancer cells are mixed up with

ordinary epithelium, it is more difficult to base a diagnosis upon such appearances, and this can only be done in conjunction with the other symptoms.

Tumours of considerable size (polypi, &c.) have been known to pass with the urine.

7. Parasites.

1. Fungi.—Adopting the same classification as before, we shall divide the fungi of the urine into moulds, yeasts, and fission-fungi, and we shall also consider them under the two headings of pathogenic and non-pathogenic organisms.

(*a.*) *Non-Pathogenic Fungi.*—Fresh healthy urine is free from fungi (*Leube*);[32] but when allowed to stand for some time, it becomes crowded with these organisms.

All three classes of fungi may be represented in the urine. It must be mentioned, however, that where ammoniacal fermentation is in progress, as a rule only fission-fungi, and perhaps a few yeast-cells, are to be found. Moulds are, under normal circumstances, a very rare manifestation in decomposing urine; but in that of diabetes, when the

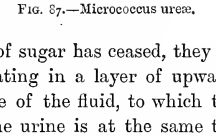


Fig. 87.—Micrococcus ureæ.

alcoholic fermentation of sugar has ceased, they make their appearance in great quantities, floating in a layer of upwards of a millimetre in thickness on the surface of the fluid, to which they impart a disagreeable mouldy smell. The urine is at the same time turbid with yeast-fungi and bacteria, and its appearance alone may be conclusive as to the abundant excretion of sugar.

The development of yeast fungi in large numbers is a sure sign of sugar in the urine, and their detection will serve to suggest this condition where it has been previously overlooked.

The microscopical character of fermenting healthy urine is subject to great variety. The transformation of urea into carbonate of ammonia is most likely effected through the agency of several forms of fungi (*Miquel, v. Jaksch, Leube, Billet, C. Flügge, v. Limbeck*[33]), but most prominent are the micrococci. Of these, the Micrococcus ureæ (fig. 87) may be seen in almost pure cultivations upon the surface of the fluid. This micro-organism forms in long chain-like series, and is of comparatively large size. In addition to these, there are rod-shaped bacteria of all sizes and forms, and as occasional manifestations certain

long spiral bacilli with large spores, and cocci, which group themselves into globular masses of dark colour and varying size (fig. 88, *g*). Sarcina is also found in the urine. It is smaller than that which forms in the stomach, being in point of size comparable to the sarcina of the lung (see p. 74).

(*b*.) *Pathogenic Fungi*.—When recently-voided urine is found to contain a profusion of fungi, the condition is in every case serious, because these fungi—even though not specifically pathogenic—may give rise to serious trouble by promoting decomposition within the bladder. The circumstances which influence the development of non-pathogenic fungi in fresh urine have been made the subject of repeated investigations (*Roberts*, *Schottelius*, *Reinhold*).[34] The matter is still surrounded with a great deal of uncertainty, and it is convenient provisionally to distinguish the condition as idiopathic bacteriuria. According to the researches of *Schottelius*, it is unattended with any morbid symptoms. In the case of a patient who had suffered for a year from

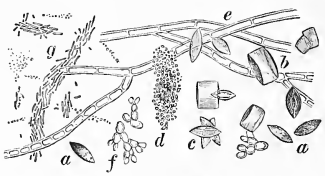


Fig. 88.—*a*, *b*, *c*. Various forms of uric acid ; *d*. Micrococci in cylinder form ; *e*. Moulds ; *f*. Yeast fungi ; *g*. Bacilli and micrococci.

gonorrhœa and cystitis, and who said that he had never had a catheter passed, the author found the urine turbid and ammoniacal, and proliferating crowds of micrococci of every description. This continued long after the cessation of symptoms, and was attended with pain.

The diagnosis of idiopathic bacteriuria must be made with great caution. The author had the opportunity of observing another case, apparently analogous to the one described above. From a communication recently made by the patient, however, the symptom is sufficiently explained by the appearance in the interim of a prostatic abscess.

Bacteriuria is very often observed after the use of unclean (not sterilised) catheters.[35] Often (although not always) cystitis supervenes as a result of this. A very interesting communication on the subject of the decomposition of urine by bacteria has been made by *Crämer*.[36]

Pathogenic fungi occur in the urine in connection with certain specific diseases, as erysipelas, relapsing fever, septic processes, typhoid, and tuberculosis, and it is a matter of great importance to be able to detect them.

In introducing this subject a few general remarks are called for :—

In the first place, there can be no doubt that large numbers of sufficiently characteristic micro-organisms may be present in the freshly-voided urine of infectious diseases, especially when it also contains albumin and casts (*Kannenberg, Litten, v. Jaksch*).[37] In one such disease, namely, erysipelas, the author's experience has been that, in all cases where the typical symptoms of acute nephritis supervened, the urine contained a profusion of fungi which were indistinguishable in their form from the Streptococcus pyogenes or erysipelatos (*Fehleisen*).[38] The urine was nearly always turbid, and while still quite fresh exhibited these bodies in moniliform arrangement. In these cases it always happened that the bacteriuria and nephritis disappeared with the cessation of the erysipelas.

That it was a true nephritis which terminated favourably in all these cases was sufficiently shown by the results of microscopical and chemical examination, which disclosed the presence of albumin, blood casts (of Groups I. and II.), renal epithelium, and numerous leucocytes in the urine.

It has been already mentioned that in septicæmic processes the urine has repeatedly been seen to hold cylindrical bodies, whose chemical properties showed them to consist of micrococci (*Martini, Litten, Senetz*).[39] *Weichselbaum*[40] has found specific micrococci in the urine in ulcerative endocarditis; *Lustgarten* and *Mannaberg*[41] cocci in acute nephritis; and *Letzerich*[42] bacilli in the "primary" nephritis of children. *Mircoli*[43] also determined the existence of pneumococci-like forms in the urine of children suffering from this disease. Further, *Neumann*[44] has found the typhoid bacillus in the urine in six out of twenty-three cases investigated; and *Philipowicz*[45] has recognised in it the tubercle bacillus and the bacillus of glanders.

The spirilla of relapsing fever (p. 30) occur very rarely, and only when hæmorrhage takes place into the kidney during the period of exacerbation; but *Kannenberg*[46] asserts that various forms of microbes are detached from the kidney in very great numbers in the exacerbations of this disease.

The recognition of tubercle bacillus in the urine has of recent years been invested with great pathological interest (*Leube, Rosenstein, Babes, Shingleton Smith, Irsai, Benda, Kreske*).[47] The method of its detection is the same as that already described in connection with the sputum (p. 75). Its presence in general points to tubercular ulceration in some part of the urinary tract, and most unequivocally when the bacilli are found to be arranged in S-shaped aggregations (fig. 89), or in colonies of unmixed constitution (pure cultivations). It should be stated, however, that *Philipowicz*[48] has discovered isolated specimens

of the bacillus in the urine in miliary tuberculosis, where there were no tubercular ulcers in the genito-urinary passages. The localisation of ulcerated patches must depend upon the other microscopical constituents of the urine. When these point to an affection of the kidney, we are warranted in inferring tuberculosis of that organ.

A form of caseation is known to occur in the kidney which, in its gross appearances, closely resembles chronic tuberculosis. And in this condition a careful examination, whether of the urine or of the caseous masses removed from the organ after death, will fail to reveal the specific bacillus. It would appear, therefore, that chronic non-specific inflammatory changes, such as we are familiar with in the lungs, may take place also in the kidney, and lead to destruction of its tissues.

When, in the course of pulmonary tuberculosis, the urine is found to contain albumin or pus, it will suggest to the physician the possibility of the renal complications which are known to attend the diathesis; he will diligently examine the urine for the tubercle bacillus, when the symptoms, after microscopical, chemical, and clinical investigation, do not find their explanation in the assumption of amyloid degeneration of the kidneys, of chronic nephritis, or cystitis.

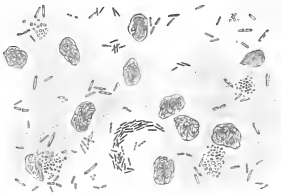


Fig. 89.—Tubercle Bacilli from the Urine.

Actinomyces may also appear in the urine in cases where the genito-urinary tract is infested with it, or when it has discharged thereby from other parts.[49]

In searching for pathogenic fungi, it is essential that the parts about the meatus be carefully cleansed, and the urine passed into a thoroughly disinfected vessel.[50] It should then be allowed to settle, and cover-glass preparations made from the sediment in the usual manner. In some cases it will be necessary to resort to Koch's method of plate-cultivation to obtain the various micro-organisms in an unmixed condition. Finally, the inoculation of animals will resolve doubt as to the character of the specific organisms obtained.

2. Infusoria.—The author has frequently observed infusoria in the urine, but never when it was fresh. They made their appearance in all cases when it had been allowed to stand for some time, and the fluid

containing them was generally feebly alkaline. Amongst these organisms were bodies which were similar to the cercomonad already described in the chapter on *Fæces*. *Hassal*[51] has given to one of the infusoria of the urine the name of Bodo urinarius.

The presence of infusoria has no pathological significance. *Bälz*[52] observed a great quantity of amœbæ in the turbid urine of a girl twenty-three years of age who was affected with phthisis. These were of larger size than the forms already described as occurring in the stools.

3. **Vermes.**

1. **Distoma Hæmatobium.**—The eggs of this parasite are often found both in the urinary passages and in the urine of inhabitants of the Tropics (see p. 35). In such cases the latter furnishes other evidence of the parasite, as blood, and frequently abundance of fat. The condition is attended with severe burning pains in micturition.[53] These

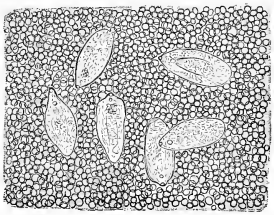


Fig. 90.—Distoma Hæmatobium from Urinary Sediment.

are momentary, and are caused by the passage of the eggs (see p. 36) along the urethra, which they irritate with their sharp angles. With the last drops of urine a blood clot is often passed. The fluid is usually clear, and contains blood cells and pus, amongst which the eggs represented in fig. 90 are to be sought.

2. **Filaria Sanguinis Hominis.**—Lewis has detected filaria in the urine in some cases in which the blood was much infested by it (fig. 21), and its appearance was generally accompanied with blood and pus. It is most likely this worm which causes the tropical hæmaturia of which *Wucherer* first sent an account from Brazil.

3. **Echinococci.**—The hooklets and fragments of the cysts of echinococcus occur as a very rare manifestation in the urine (*Mosler*).[54] In such cases, the cysts may have formed originally in the urinary passages—and this is very exceptional—or they may have found their way into them by rupture from a neighbouring organ. When the hooklets and characteristic membrane (fig. 45) are present, the sediment is likely also to contain red blood-corpuscles, numerous leucocytes, and a quantity

of cellular débris from that part of the urinary apparatus which has been injured by the separation of the cyst.

4. Eustrongylus Gigas.—This parasite has been said to exist in the urine, but the researches of *Leuckart*[55] have thrown doubt upon the matter. At all events, it must be very rare.

5. Ascarides.—In exceptional instances ascarides make their way from the intestine into the urinary passages. Their presence in the urine is usually brought about by an abnormal communication between the latter and the alimentary canal. *Scheiber*[56] has recently found in the urine of a woman worms which he thought had been derived from the genital organs, and he has named them Rhabditis genitalis. Similar observations have been reported by *E. Peiper*, *Westphal*, and *Baginsky*.[57]

II. CRYSTALLINE AND AMORPHOUS DEPOSIT (UNORGANISED SEDIMENT.)—It will be convenient in treating of the inorganic deposit of the urine to consider, in conjunction with the microscopical appearance of its constituents, some of the more characteristic of their chemical and histo-chemical properties.

The colour of the sediment and the reaction of the urine will often afford some indication of the nature of the deposit. Thus, if the sediment of a deep red colour forms when the urine has stood for a short time, it consists mainly of urates. The colour is then due to the presence of urinary pigments, carried down by the deposit, uric acid and its salts being themselves colourless. If the deposit redissolves on the application of heat *without the addition of acid*, it is a further proof that it is composed of urates.

If, on the other hand, the urine is alkaline, and deposits a white flocculent sediment, the latter probably either consists of pus or contains a large proportion of phosphates, carbonates, and alkaline urates. Such a sediment is *insoluble* by heating, but readily so in presence of acid (acetic acid).

A third kind of sediment, of a mixed character, may be distinguished. This consists of urates and phosphates, and it forms in urine which was acid when passed, but which has become gradually alkaline from the supervention of ammoniacal fermentation.

An abundant sediment of urates belongs to the urine of fever and renal congestion, and occurs also in healthy individuals after excessive perspiration without partaking freely of water (see p. 171). It is thought by *Mygge*[58] that the presence of such sediment, when it consists of uric acid, has a certain clinical significance, inasmuch as it is generally found in cases of rheumatism or renal disease.

Phosphatic sediment is apt to form in all conditions where the urine

possesses an alkaline reaction when passed. It does not, however, always indicate disease, because it may be induced by the drinking of aerated water and by other means. Amongst pathological conditions dyspepsia often occasions the deposit of phosphates, and in general it may be said that phosphatic sediment is associated with chronic, as distinguished from acute, affections.

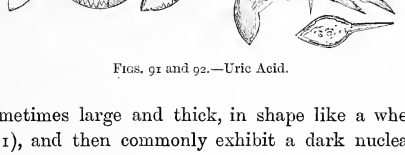
The characters above referred to will serve to indicate which class of salts preponderate in the urine. A more accurate estimation of its inorganic constituents can be formed only from a microscopical and histochemical investigation of the sediment.

Such constituents may be *crystalline* or *amorphous*. Their nature will further differ according as they precipitate in an acid or an alkaline urine. We shall, therefore, consider the sediments of acid and of alkaline urine separately.

A. Sediments of Acid Urine.

(*a.*) Crystalline Deposits.

1. Uric Acid.—Uric acid occurs in crystals, which are deeply stained a brownish-yellow colour, and differ much as to their form and size.



Figs. 91 and 92.—Uric Acid.

They are sometimes large and thick, in shape like a whetstone (figs. 88, *a*, and 91), and then commonly exhibit a dark nucleated centre; sometimes they are longitudinally striated spicules (fig. 92), and sometimes again rhombic tables (figs. 88, *b*, and 91) with rounded angles. They may appear separately or in masses. Their shape and size vary greatly, their most characteristic property being their colour, and through it alone they may be readily recognised. They may be seen under the microscope to dissolve in caustic potash, and can again be made to crystallise in the rhombic form by neutralisation with hydrochloric acid. The murexide test (see p. 47) may be employed in appropriate cases for their detection.

2. Oxalate of Lime.—This substance crystallises in transparent, strongly refracting octahedra (envelope crystals), which are readily soluble in hydrochloric and insoluble in acetic acid (*Fürbringer*).[59]

The occurrence of isolated crystals of oxalate of lime (fig. 93) has no clinical significance. They have been found in healthy urine, and their number may be greatly increased after the ingestion of food

containing a large proportion of oxalic acid, such as tomatoes, fresh beans, beetroot, asparagus, &c. On the other hand, oxaluria as a morbid state cannot be measured by the use of the microscope alone, since the urine may contain a large proportion of oxalic acid, whilst neither it nor its salts are precipitated as crystals. In such cases, the urine must be analysed with a view to ascertaining the proportion of oxalic acid which it contains.

3. Bilirubin and Hæmatoidin.—*Bilirubin* is deposited in the urine either in the amorphous form or in crystals. Crystals of bilirubin have a twofold character, occurring either as clusters of needles, or as minute rhombic tablets, and their colour ranges from yellow to a beautiful ruby-red. They are soluble in caustic soda, and on the application of a drop of nitric acid a green rim forms round them. *Kussmaul*[60] has discovered these crystals in jaundice, and *Ebstein*[61] in pyelonephritis.

Hæmatoidin resembles bilirubin as closely in appearance as in its

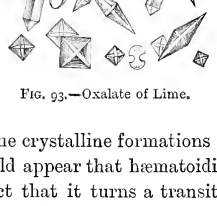


Fig. 93.—Oxalate of Lime.

chemical properties. The crystalline formations of the two are identical (see fig. 71); but it would appear that hæmatoidin may be distinguished chemically from the fact that it turns a transitory blue when treated with nitric acid (*Holm*),[62] and is insoluble in caustic potash and æther (*Städeler*).[63] According to *Hoppe-Seyler*,[64] hæmatoidin and bilirubin are in all respects indistinguishable, and the experience of the author lends support to the view of this great authority; for he has repeatedly had occasion to observe that, in the urine of jaundice, the yellow cellular elements, and notably the epithelium, are stained red, passing to blue on the application of nitric acid—a reaction which is assumed to belong only to hæmatoidin, and yet in these cases there can be no doubt that the substance present is bilirubin (see also p. 39).

Leyden[65] found these crystals in nephritis gravidarum, and *Fritz*[66] in various chronic and acute diseases, *e.g.*, in a case of carcinoma of the liver, in scarlatina and typhoid. They were usually connected with cellular débris, and in jaundice alone were they also met with in the free state. In general it may be said that the presence of such free

crystals in considerable quantity implies an antecedent hæmorrhage or the bursting of an abscess—as a suppurating echinococcus cyst—into the urinary passages.

4. Triple Phosphates.—Crystals of triple phosphate occur commonly

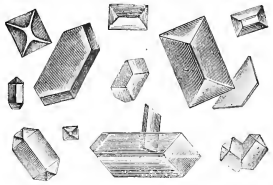


FIG. 94.—Crystals of Triple Phosphate.

in weakly acid urine, as in the fæces (see p. 157), as bodies of large size and of the coffin-lid form (fig. 94). They are readily soluble in acetic acid. Even when found in large numbers, this fact alone will hardly warrant the diagnosis of phosphaturia.

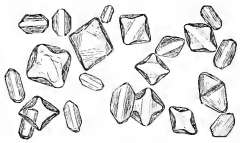


FIG. 95.—Crystals of Basic Magnesium Phosphate.

5. Basic Phosphate of Magnesia.—The crystals of this body have the form of large strongly refracting plates, usually in the shape of elongated rhombic tablets (fig. 95). They are readily soluble in acetic acid, and precipitated again on the addition of carbonate of soda. They

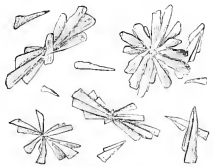


FIG. 96.—Crystals of Neutral Calcium Phosphate.

are found in concentrated urine of feebly acid, neutral, or alkaline reaction (*Stein*).[67]

6. Neutral Phosphate of Lime.—These crystals appear as pointed wedge-shaped prisms, either singly or in clusters. They are decom-

posed by ammonia, and dissolve in acetic acid (fig. 96). They occur commonly in feebly acid urine which is becoming alkaline.

7. Sulphate of Calcium.—This substance is rarely present in the urinary sediment. It generally takes the form of long colourless needles, but occurs also in elongated tables with abrupt extremities. Amongst such crystals are sometimes to be seen masses of indeterminate crystalline structure (fig. 97). They are insoluble in ammonia

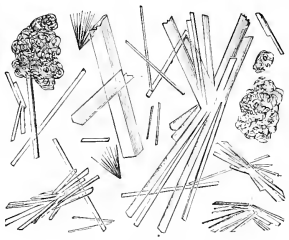
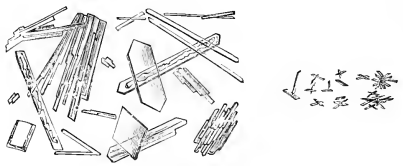


FIG. 97.—Sulphate of Calcium.

and acids. Clinically their presence is of little consequence (*Valentiner Fürbringer*).[68]

8. Hippuric Acid.—Crystals of hippuric acid have the form of four-sided prisms, and are scattered separately or in groups (figs. 98 and 99) through the sediment.

They are soluble in ammonia, insoluble in hydrochloric acid. They occur in considerable quantity after the exhibition of benzoic acid, or



FIGS. 98 and 99.—Crystals of Hippuric Acid.

the ingestion of certain fruits, as cranberries and bilberries. Otherwise they are a rare manifestation, and have little influence on diagnosis.

9. Cystin.—The crystals of this body are seen as symmetrical hexagonal tables (fig. 100, *b*), superimposed upon or contiguous to one another. They are insoluble in acetic acid, but readily soluble in ammonia. In this they differ from the crystals of uric acid.

Cystin may also be present in solution in the urine. It may then be precipitated by acetic acid.

When crystals are found in the urine which answer to the description given here, they should be separated by filtration or decanting, the sediment washed with a little water, and the substance in question tested on the platinum foil. Cystin burns with a bluish-green flame, and without melting.[69]

If cystin be boiled in caustic potash holding oxide of lead in solution, sulphide of lead forms (*Liebig*).[70]

When heated with caustic potash on a silver plate (as a coin), it leaves a brown or black permanent mark. When dissolved in a boiling solution of caustic potash, dilution with water and the addition of a solution of ferrocyanide of sodium give a violet colour (*J. Müller*).[71]

This reaction, according to *Krukenberg*,[72] belongs not to the cystin, but to sulphide of calcium, which constitutes an impurity of the caustic potash with which it has been boiled.

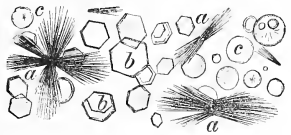


Fig. 100.
a. Tyrosin; *b*. Cystin; *c*. Leucin.

10. Xanthin.—*H. Bence Jones*[73] once found this substance in the urine of a lad who had three years previously exhibited the symptoms of renal colic. It was seen in the sediment in the form of whetstone crystals, which were insoluble in acetic acid and soluble in ammonia (thus distinguished from uric acid). Such bodies are clinically of importance, inasmuch as they may give rise to the formation of calculi (see the observations of *Bence Jones*, l.c.).

11. Tyrosin and Leucin.—These substances generally occur together in the urine.

(*a*.) *Tyrosin* is seen in the sediment in sheaves of very fine needles (fig. 100, *a*), which are insoluble in acetic acid, soluble in ammonia and hydrochloric acid.

To determine the character of tyrosin chemically, the sediment containing it should be separated by filtration, washed with water, dissolved in ammonia with the addition of carbonate of ammonia, and the solution allowed to evaporate. The tests for tyrosin may then be applied:—

1. A milligram of the substance obtained is placed on a watch-glass

and moistened with a drop or two of sulphuric acid. The mixture is covered and allowed to stand for half-an-hour. It is then diluted with water, heated, while still hot saturated with calcium carbonate, and filtered. The filtrate is colourless, and when treated with acid-free ferric chloride, it assumes a violet tint (see p. 117), (*Piria, Städeler*).[74]

2. When the tyrosin is heated with nitric acid on platinum foil, it assumes an orange-yellow colour. The residue is dark yellow, and turns reddish-yellow on the addition of caustic soda. When the latter is evaporated, the substance which remains is of a deep brownish-black colour (*Scherer*).[75]

3. Tyrosin crystals are dissolved in hot water, and the still hot solution is treated with mercuric nitrate and nitrite of potash. The fluid assumes a dark red colour, and yields an abundant red precipitate (*R. Hoffmann* and *L. Meyer*).[76]

4. *C. Wurster*[77] recommends the following process:—Tyrosin is dissolved in boiling water and a little dried chinon is added. The fluid quickly assumes a deep ruby-red colour, which changes to brown after the lapse of twenty-four hours. The chinon-tyrosin reaction can be depended upon only when tyrosin has been isolated as the free acid [para-hydro-oxyphenol-amido-propionic acid], and will not serve as a test unless it appears readily after the application of heat without prolonged boiling. Chinon alone, or in presence of phenol, gives, when boiled, a rosy-yellow colour to its solutions.

Tyrosin occurs dissolved in the urine as well as in crystals. To obtain it from solution, basic acetate of lead should be added, when a precipitate will form. The fluid should now be filtered, and the filtrate freed from lead by the addition of sulphuretted hydrogen, again filtered, and partially evaporated on the water-bath. The residue is repeatedly extracted with small quantities of strong alcohol, and the extract several times boiled with weak alcohol and then allowed to evaporate spontaneously.

(*b.*) *Leucin.*—This body, which is commonly associated with tyrosin, is for the most part held in solution in the urine, but to some extent occurs also in the sediment in the form of small spheres (fig. 100, *c*). The process for its detection is the same as that for tyrosin. It may be separated from the latter by crystallisation from a watery solution, and then purified by recrystallisation from a solution of ammonia in boiling alcohol. When quite pure, leucin crystallises in delicate plates; when impure, it forms little bulbs of amorphous structure. It may be known by the following tests:—

1. A solution containing leucin, when heated with proto-nitrate of mercury, deposits metallic mercury (*Hofmeister*).[78]

2. When consumed with nitric acid on platinum foil, it leaves a

colourless residue. This, when heated with caustic potash, forms drops of an oily fluid which does not adhere to the platinum (*Scherer*).[79]

Tyrosin has been found in the urine in conjunction with leucin in phosphorus poisoning, acute yellow atrophy of the liver, and several of the infectious diseases (*Frerichs, Schultzen, Riess, Pouchet, A. Fränkel, Blendermann, A. Irsai*).[80]

It is probable, however, that in some instances where these bodies were supposed to have been in the urine they were not sufficiently identified; and it is within the author's experience that a deposit has repeatedly been taken as consisting of tyrosin, until subsequent chemical analysis exposed the error. *Prus*[81] has found abundance of leucin in the urine in cases of leukæmia.

12. Soaps of Lime and Magnesia.—In the urine of various diseases are often found crystals bearing a very close resemblance in form to

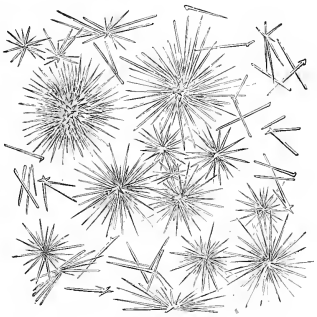


Fig. 101.—Soaps of Lime and Magnesia from the Urinary Sediment.

those of tyrosin (*v. Jaksch*), but possessing distinctive characters of their own. The accompanying illustration (fig. 101) represents such crystals, which the author had the opportunity of examining once only in tolerable abundance. They occurred in the sediment from the feebly acid urine of a woman with severe puerperal septicæmia. They are obviously very similar to those of tyrosin, but yielded none of the characteristic reactions of that body as given above (1–3). The material was not sufficient for further analysis; but it appeared from the behaviour of the substance in question with regard to solubility, &c. (see p. 156), that it was probably formed of the lime and magnesia salts of the higher fatty acids.

(*b.*) Amorphous Deposits.

1. Urates.—Amorphous urates have the appearance of fine granules, disposed singly or in masses. They are entirely dissolved by heating

or the addition of acids, and the sediment when treated in this way exhibits free uric acid, for the most part in the form of rhombic tablets.

2. Oxalate of Lime.—The envelope crystals of oxalate of lime have been already described (see p. 157). This substance appears also as dumb-bell-shaped figures. They are unaffected by acetic acid, and dissolve in concentrated solution of hydrochloric acid.[82]

3. Sulphate of Calcium.—In addition to its crystalline form (p. 198 and fig. 97), sulphate of calcium in the urine takes the appearance of dumb-bell-shaped amorphous masses, which are insoluble in ammonia and in concentrated solutions of hydrochloric acid.

When the sediment contains this substance in considerable quantity, it may be separated from the other constituents by decanting, filtering, and washing in cold water. It is then dissolved in a large bulk of hot water. If to one portion of this solution a quantity of chloride of barium be added, a precipitate of barium sulphate forms, and this is insoluble in nitric and in hydrochloric acid. If another portion be treated with ammonium oxalate, a precipitate of oxalate of lime falls. This precipitate is insoluble in acetic and soluble in hydrochloric and nitric acids.

4. Brown and Yellow Concretions.—The urinary sediment may contain such concretions either free or associated with cells, and consisting of hæmatoidin or of bilirubin, which, as we have seen, is perhaps indistinguishable from hæmatoidin. According to *Holm*,[83] when a substance of this sort is found to be soluble in caustic potash, and exhibits a coloured ring, of which green forms one zone, on the application of nitric acid, we are to assume that it consists of bilirubin. When, on the other hand, it is insoluble in caustic potash, and colours transitorily blue with nitric acid, he would suppose that it is formed of hæmatoidin.

5. Fat.—Fat is deposited in the form of strongly refracting globules of varying size, which are readily soluble in æther. It may be present in the urine in small quantities after the fracture of bones, and in chronic inflammation of the kidney, attended with much fatty degeneration of that organ. It occurs in greater abundance, however, only in chyluria, which for the most part depends upon the action of certain worm parasites (Distoma hæmatobium and Filaria sanguinis hominis), and in phosphorus poisoning. We shall have occasion later to revert to the subject of chyluria and lipuria.

B. **Sediments from Alkaline Urine.**

(*a.*) **Crystalline Deposits.**

1. Triple Phosphate.—The crystals of triple phosphate deposited in alkaline urine exhibit a very great diversity of character, especially

when newly formed in the process of ammoniacal fermentation. They may be seen under such circumstances to resemble snowflakes, or again as peculiar jagged figures, resembling flags or elder leaves (figs. 94 and 102). Their most permanent and characteristic form, however, is that of large colourless and more or less perfect coffin-lids or knife-rest form.

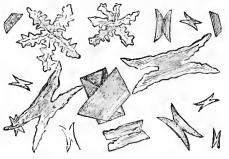


Fig. 102.—Crystals of Triple Phosphate.

2. Indigo.—Indigo occurs in the urine as concretions and amorphous fragments, and also in the form of blue crystals and fine blue needles, which mostly cohere in clusters. Crystals of indigo are no very rare manifestation in decomposing and fermenting urine. They

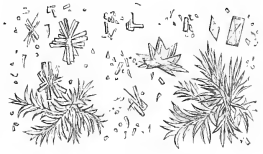


Fig. 103.—Indigo Crystals.

are derived from the decomposition of sulphate of indoxyl. This substance was found to be present in remarkable abundance during the process of ammoniacal fermentation in the urine of jaundice, from a patient with hypertrophic cirrhosis of the liver. The urinary sedi-

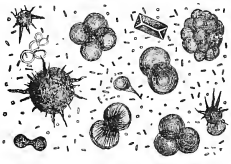


Fig. 104.—Urate of Ammonia.

ment, in a case of abscess of the liver, was recently examined by the author. It contained numerous indigo crystals, and had an *acid* reaction (fig. 103) (*v. Jaksch*).

3. Urate of Ammonia.—Urate of ammonia forms spherical bodies,

dark in colour and of varying size. Their circumference is beset with radiating spicules of crystalline structure (fig. 104). They are soluble in hydrochloric and acetic acid, with the formation of uric acid, which is deposited in the form of rhombic tables.

4. Phosphate of Magnesia.—The crystals of this salt have been already (fig. 95) sufficiently described.

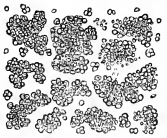


FIG. 105.—Carbonate of Lime.

5. Cholesterin.—Crystals of cholesterin are very rarely to be seen in the urinary sediment. The author has observed them but once, and that was in the case of a man who suffered from tabes and cystitis. The cholesterin took forty-eight hours to deposit in crystals. The urine when freshly voided had a slightly acid reaction, was turbid, and when shaken, a great number of scaly particles could be seen in it with the naked eye (fig. 106).

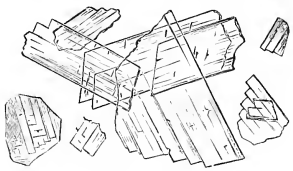


FIG. 106.—Cholesterin.

(*b.*) Amorphous Deposits.

1. The large, dark-coloured, spherical bodies represented in fig. 104 consist of *urate of ammonia*. They are distinguished by their solubility in acetic and phosphoric acids, with the simultaneous deposit of rhombic tables of uric acid.

2. Larger or smaller particles, which dissolve in acetic acid without the evolution of gas : *basic phosphatic earths.*

3. Particles of varying size, which are soluble in acetic acid with the evolution of gas : *carbonates of the alkaline earths.*

4. Dumb-bell-shaped masses and coarsely granular concretions, dissolving in acetic acid with the evolution of gas : *carbonate of lime* (fig. 105).

5. *Indigo.*

III. Urinary Concretions.

Concretions of considerable size are occasionally to be seen with the naked eye in the urine (urinary sand). They consist for the most part of urates, or of urates and uric acid together. Their recognition is of great consequence in the diagnosis of renal colic (nephrolithiasis). These concretions are usually more or less stained, and their constitution may be readily determined by the tests already given for the compounds of uric acid (p. 195).

Phosphatic concretions of larger size occur more rarely. They are light-coloured and of little consistence.

Other concretions occasionally to be met with in the urine are those of cystin, xanthin, oxalic acid, and indigo (*Ord*, *H. Chiari*).[84] Those of the last-named substance are easily recognisable by their colour. The constitution of these bodies is dealt with at greater length in the ordinary text-books on the chemistry of urine (see *Huppert*, *Hoppe-Seyler*, and *Leube-Salkowski*).

IV. Foreign Bodies in the Urine.

As occasional impurities of the urine, may be enumerated fatty particles (often introduced with the catheter), fibres of silk, linen, and wool, particles of feathers and wood, and starch granules, the latter being employed as starch powder in medication of the urethra.

The appearance in the urine of substances belonging to the fæces is a fact of serious import. In drawing a conclusion from their presence, care, of course, must be taken to ascertain that they were actually passed with the urine; and when this is so, they are evidence of an abnormal communication between the urinary passages and the gut.

Fragments derived from tumours, as sarcoma and carcinoma, may find their way into the urine by invasion from neighbouring organs (see p. 192).

Hairs have been known to be passed with this fluid (pilimictio). They have generally been derived from dermoid cysts in the urinary passages. Sometimes they have been conveyed by accident or design (hysteria) into the recipient vessel. In very rare cases gases are voided with the urine (pneumaturia) in considerable quantity; but when this has occurred, there has, as a rule, been an abnormal communication between the urinary passages and the intestine (see *Hydrothionuria*). It must, however, be borne in mind that gas may be freely generated in the bladder by spontaneous decomposition of the urine.

III. CHEMICAL EXAMINATION OF THE URINE.

A. Organic Substances.

1. Proteids.—We shall begin with the consideration of albumin, which is the commonest of all the morbid constituents of the urine.

It is still an open question whether albumin ever occurs in considerable quantity in the urine of health. For whilst, on the one hand, the older authorities (as *Frerichs*, *Vogel*, and *Ultzmann*[85]) have recorded its occasional presence in healthy urine, and the more recent investigations of *Leube*, *Fürbringer*, *Senator*, and *C. Posner*[86] would seem to have established the possibility of a physiological albuminuria, the researches of *v. Noorden* are not less decisive in the opposite direction; and *Leube*[87] from recent very careful experiments concludes that not *every* urine contains albumin. [See Appendix XVIII.]

For the purpose of such investigations the following plan may be adopted:—Normal urine is taken, and it is first ascertained to be free from bacteria and from albumin, as shown by the ordinary methods. It is next distilled in vacuo at a low temperature (35° to 37° C.), and the residue, with the included sediment, is either tested directly, in the manner to be indicated at p. 210, or it is first treated with alcohol; and of the sediment which remains when the spirit has been driven off, one part is dissolved in water, another in acetic acid, and a third in caustic potash, and the solutions submitted to the tests described at p. 210 (*Anschütz*).[88] Proceeding in this way, it will be found that *Leube's* statement concerning healthy urine is entirely correct, and not a trace of albumin can be shown in it. In the urine of certain diseases, on the other hand, as in valvular heart-disease with compensating hypertrophy, where the most sensitive tests directly applied will fail to evince the presence of albumin, it can readily be shown in the concentrated fluid obtained as above.

It may at all events be confidently stated that the urine occasionally holds a variable quantity of albumin as a temporary constituent, whilst at the same time the kidneys exhibit no alteration of structure, and its presence in such cases is to be attributed to a sudden disturbance of the circulation (see *Schreiber's* experimental albuminuria). To the same category of causes must be ascribed the conditions similarly observed by *Stirling* in apparently healthy boys, and by *Pavy*.[89] Albuminuria of the like cause and import sometimes occurs, as *Falkenheim*[90] has remarked, in connection with morbid states. *Virchow* originally, and after him many other observers, have shown that the urine of newly-born infants often contains albumin.[91]

Bright[92] in England was the first to determine the connection between renal disease, dropsy, and albuminous urine, and after him the labours of such men as *Christison* and *Rayer*, *Frerichs* and *Traube*,[93] formulated what was known of albuminuria as a clinical symptom. For a long time it was thought sufficient merely to determine the presence of proteid matter in the urine, and the further question was not raised as to whether it occurred in more forms than one. It is now established on clinical and physiological grounds that, in addition to serum-albumin, the urine may contain globulin, peptone, albumose, oxyhæmoglobin, mucin, and fibrin; and it is obviously important to be able to distinguish between these bodies.

At present, the chief clinical interest attaches to serum-albumin, peptone, and albumose, for by methods now well established we can readily separate each of these from the others. The very simple methods which *Kauder* and *Pohl*[94] (of Hofmeister's laboratory) have recently communicated for the detection of globulin in serous fluids and urine have placed us in the same favourable position with reference to that body, and by their aid we may hope soon to learn whether globulinuria also exists as an independent condition. In view of their importance in this connection, we shall describe these methods with the others. It follows from what has been said that albuminuria is of different kinds, and we may distinguish : (1) Serum-albuminuria, which alone will be understood to be meant by the term "albuminuria" in the following pages; (2) Peptonuria; (3) Albumosuria; (4) Globulinuria, which as yet is not known to occur by itself; (5) Fibrinuria; (6) Hæmaturia (see p. 222); (7) Hæmoglobinuria; and (8) Mucinuria.

1. **Albuminuria.**—Under this heading we shall group those states in which the urine is found to contain serum-albumin, in combination, perhaps, with a variable proportion of globulin.

The author has come to the conclusion, as the result of repeated analysis, that the urine of serum-albuminuria does not invariably contain globulin.

Serum-albumin in notable quantity is never found in healthy urine. Its appearance is in all cases a morbid symptom of great importance.

The albumin of urine may be derived from the kidney (renal albuminuria), or by admixture from parts in the urinary passages beyond the kidney (contingent, accidental albuminuria).

(*a.*) **Renal Albuminuria.**—This form of albuminuria, which is at once the commoner and the more serious, depends in all cases upon disturbance of the renal functions, and such disturbance may be due to various causes. By far the most frequent of these are inflammatory and degenerative changes in the structure of the kidneys. It should be mentioned here that where such processes in the kidney are the underlying cause of albuminuria, we cannot always infer their extent and severity from the quantity of albumin eliminated. So far indeed is this from being the case, that there are certain forms of renal disease of an especially serious character (granular kidney, red atrophy), in which the urine contains only traces of albumin. Another cause of albuminuria is found in disturbances of the circulation of different kinds, provided only that such disturbance extends to the vessels of the kidney. It must, however, be borne in mind that errors of the circulation, when of long continuance, may effect changes in the structure (congestion) of the kidney.

To disturbances of the circulation should perhaps be referred the

temporary albuminuria of epileptic paroxysms (*M. Huppert*[95]), and that which *Schreiber*[96] induced experimentally by compression of the thorax in healthy persons. Possibly, also, that form of albuminuria which often sets in in acute gastritis should be mentioned here (*Singer*).[97] As a permanent condition referable to renal congestion may be mentioned the albuminuria of emphysema, heart-disease, weakened heart, &c.

To a third class of causes is to be ascribed the albuminuria of fever (*Leyden*).[98] The circumstances which promote the elimination of albumin in the febrile state are manifold. In the first place, the accompanying changes in blood-pressure would be alone sufficient to account for albuminuria. Then it is known that a long continuance of the febrile processes is competent to induce structural alterations in the renal epithelium, which in their turn lead to albuminuria. It is conceivable, again, that the special cause (fungi) in certain fevers exerts an immediate influence in the matter, since we find that in many infectious diseases the proliferating micro-organisms are eliminated in large quantities by the kidneys (see p. 190). The admirable experiments recently undertaken by *H. Lorenz*[99] lend great support to the theory that the albuminuria of fever depends directly upon certain histological changes in the renal epithelium, especially affecting the "rodded" lining cells.

There is a fourth form of albuminuria to be distinguished in respect of causation from those already mentioned. It occurs in anæmic and enfeebled individuals, independently of kidney disease, disordered circulation, and the febrile state; and can be accounted for only by assuming changes in the constitution of the blood, of such a character that, whilst the structure of the kidneys remains intact, and the blood-pressure is not appreciably altered, albumin is allowed to exude with the urine (*v. Bamberger's* hæmatogenic albuminuria).[100]

It still remains to say a few words regarding that variety of albuminuria which is intermittent in character. In the author's experience it occurs under the most varying circumstances, and may be either renal or accidental (*q.v.*) in its origin. A number of observations of the kind recently published by *Buil*, *Mareau*, *Klemperer*, *Caufield*, and *G. Johnson*[101] have proved its occurrence in health, where, no doubt, it is due to the circulatory disturbances already referred to.

In the course of a chronic or of an acute nephritis, it will often happen that albumin is found to be temporarily present in the urine (*v. Jaksch*).[102] When this is so, a careful microscopical examination of the fluid, at a time when it is *free from albumin*, will usually disclose the presence of formed material (casts and renal epithelium) by which the existence of a nephritis may be known. Such intermittent albuminuria belongs especially to contracted kidney. In a case of this

disease, if the quantity of urine passed in the twenty-four hours be collected together and examined, it will nearly always be found to contain albumin; but if the same urine be examined in separate portions at shorter intervals—say of one or two hours—it will be found comparatively often that that which is collected in the forenoon is devoid of albumin. But it is not in connection with kidney-disease that intermittent albuminuria is most apt to occur. It is more often associated with affections of the ureters and urethra, and especially with chronic inflammation of the prostatic portion of the latter. In this connection the urine first passed in the morning is generally turbid, and contains albumin, derived no doubt from the pus cells in the fluid.[103] *Falkenheim*[104] has recorded a remarkable case of intermittent albuminuria, caused by the pressure of a tumour on the left kidney. In *Pavy's* disease, it is probable that a careful microscopical examination would show evidence of an underlying nephritis (see *Merley*).[105] From what has been said above of the various forms of renal albuminuria, it will be seen that this condition is in itself a symptom of very ambiguous significance. Taken with the other physical and microscopical appearances of the urine—but only in conjunction with these—it will afford a valuable indication of kidney-disease. It must be borne in mind that *we are never warranted in inferring the existence of a renal affection or of a nephritis from the mere fact that the urine contains albumin*. This was the error of former times.

(*b.*) **Accidental Albuminuria.**—The appearance in the urine of albumin derived from a source other than the kidneys is a symptom of much slighter consequence. It may come from the renal pelvis, the ureters, the bladder, or the urethra, or through an abnormal communication from neighbouring parts, *e.g.*, the lymphatics or thoracic duct. In general, a positive judgment as to its source may be formed from the result of chemical and microscopical investigation. Thus, for instance, if the urine exhibits but little serum-albumin and a considerable proportion of pus cells, the inference is that the former is obtained from the migration of leucocytes in some part of the urinary passages just mentioned. The absence of renal casts and epithelium, moreover, goes far to exclude the possibility of renal albuminuria.

Determination of Albumin (Serum-Albumin).

(*a.*) **Qualitative Tests.**—The tests at our disposal for the estimation of albumin are very numerous. The reactions which we shall describe here are all more or less to be depended upon; but there are some amongst them which will engage a larger share of our attention, and they are those which years of clinical experience have shown to be especially reliable. Moreover, when applied in the order in which they are given, they will afford a tolerably accurate means

of discriminating the various forms in which albumin occurs in the urine.

1. *Nitric Acid and Heat Test.*—A portion of the urine is boiled, and a small quantity ($\frac{1}{10}$ to $\frac{1}{20}$ its volume) of nitric acid (sp. gr. 1.18) added to it. Should a precipitate form on boiling, this may consist either of albumin or of phosphates. If it dissolves on the addition of acid, it is composed of phosphates; if not dissolved in presence of the acid, and if increased thereby, it is albumin (*acid albumin*).

The application of this test, however, is open to certain fallacies. In the first place, if there be but little albumin present, the quantity of acid usually added will be relatively in excess; and under these circumstances the albumin may combine with the acid to form a soluble nitrate when no precipitate remains. Where, on the other hand, both phosphates and albumin are present, if the acid employed be too little, a portion only of the basic phosphates may be changed into the corresponding acid salts, while the albumin enters into combination with the rest, and remains in solution as an albuminate (union of albumin with a base).

Another occasional source of error is the formation of a precipitate of uric acid. Such a precipitate, however, can usually be known by its deep brown colour, and from the fact that it is never flocculent. Moreover, it does not fall until the specimen begins to cool.

Finally, the test may be misleading in cases where the urine contains a considerable quantity of resinous acids (pine acids), as happens, for instance, after the use of copaiba balsam. These acids (pinic and pimaric acids) are then precipitated by heat, but can be readily distinguished from albumin by their solubility in alcohol.

The heat and nitric acid test will serve for the detection of *serum-albumin*, *globulin*, and, where the precipitate falls on cooling, *albumose*, but not for that of peptone.

2. *Acetic Acid and Ferrocyanide of Potassium Test.*—The urine is filtered, and to the clear filtrate a large quantity of acetic acid (sp. gr. 1.064) and a few drops of a 10 per cent. solution of ferrocyanide of potassium are added. If albumin (serum-albumin) be present in considerable quantity, a flocculent precipitate forms; when merely a trace, the fluid becomes turbid or slightly opalescent.

In the latter case, it may be necessary to compare it with the filtered urine, in order to estimate the change in its appearance. It will also happen, especially when it contains micro-organisms, that the fluid cannot be rendered clear even by repeated filtration; and here again the comparison will lie between the simply filtered fluid and that to which the reagents have been added. In the first instance a minimal turbidity, in the second an increase, shows the presence of albumin.

This is a most reliable test, and by its means very minute quantities of albumin may be detected. The best and most accurate results, however, may be obtained from its application in the following manner:—A mixture containing a few cubic centimetres of fairly concentrated solution of acetic acid and a little ferrocyanide of potassium is freshly made in a test-glass, and carefully poured upon the surface of the clear filtered urine in a test-tube. If the merest traces of albumin be present, a white ring forms at the point where the two fluids are in contact. For ferrocyanide of potassium platinocyanide of potassium may be substituted. The test is then just as accurate, and the advantage of this reagent is that its solution is colourless.

By this method the presence of *serum-albumin*, *globulin*, and *albumose*, but not of peptone, can be ascertained.

3. *Biuret Test.*[106]—The urine is treated with caustic potash, and a dilute solution of sulphate of copper is added, drop by drop, with a pipette. If albumin be present, the resulting peroxide of copper (a green precipitate) is dissolved, and the fluid assumes a reddish-violet colour.

This test serves for *albumin*, *albumose*, *globulin*, and *peptone*. The latter substance, however, strikes red, not violet, in the fluid.

4. *Heller's Test.*[107]—The urine is poured carefully, so as to form a layer on the surface of some nitric acid in a test-tube. At the junction of the fluids a white cloud forms in the shape of a ring if albumin be present. This test is very accurate; but it is not to be recommended for general use, because, in the case of undiluted urine, the deposition of uric acid is apt to cause a brown discoloration, which may easily be mistaken by the inexperienced for the clouding of albumin. After the use of copaiba balsam, too, a similar ring may form.

This reaction is also the basis of an admirable method for approximately estimating the proportion of albumin in the urine (see p. 213).

There are a number of other tests for albumin of a more or less accurate and practicable character, and to some of these allusion must be briefly made.

1. *Heynsius' Test.*[108]—The urine is to be rendered strongly acid with acetic acid; a few cubic centimetres of a saturated solution of chloride of sodium are added, and the mixture boiled. The presence of albumin is shown by the formation of a flocculent precipitate. Very small quantities can be detected in this way.

2. *Hindenlang's* (*Metaphosphoric Acid*) *Test.*[109]—If to urine which contains albumin a little solid metaphosphoric acid be added, a precipitate or turbidity forms. This test is very convenient, but will not suffice where only a trace of albumin occurs. The author has repeatedly failed to obtain with it any indication of this substance, while the application of the acetic acid and ferrocyanide of potassium method exhibited its presence. On the other hand, he can confirm the statements of *Penzoldt* and *v. Noorden*,[110] who maintain that a precipitate can often be obtained by this reagent with urines with which all other albumin tests yield negative results.

3. *Fürbringer's Method*[111] of testing with chloride of mercury and sodium has been shown to be very convenient, especially when the reagent is used in the

form of capsules (*Stütz's* capsules); but it has no other advantage over those previously described. The use of test-papers also (as *Geissler's*) in examining for albumin, which has of recent years come into vogue in England and on the Continent, does not seem warranted by experience.

4. *The Picric Acid Test* of *Dr. George Johnson* [112] is sensitive, but it is not altogether reliable, since this reagent will effect the precipitation of alkaloids and kreatinin (*Jaffé* [113]) in the urine. It must, however, be studied, for it has become the basis of a well-known method for the approximate estimation of the quantity of albumin in that fluid. In this connection it will engage our attention later on (p. 216).

5. *Colour Reactions.*—Finally, the albumin group yields a number of colour reactions, many of which are applicable to the purpose of detecting its members when contained in the urine. Amongst these are the *biuret* test already mentioned, and *Millon's* reaction. The *xanthoproteic* test, and the colour-reactions of *M. Schultze, Adamkiewicz,* and *Fröhde,* [114] hardly call for special mention here, since their application chemically for the determination of serum-albumin has no advantage which does not belong to the methods adopted in the text. For particulars concerning the use of sulphuric and hydrochloric acid as colour tests for albumin, the reader may consult *Liebermann* and *C. Wurster.* [115]

Millon's Reaction calls for fuller notice. If to a solution containing albumin mercuric nitrate be added, and heat applied to boiling, the further addition of potassium nitrite will cause the precipitate (if any) and the supernatant fluid to turn red. The reaction is common to all the monohydroxyl-benzol derivatives (*O. Nasse*).[116] As a test for albumin it is somewhat ambiguous, inasmuch as it can also be obtained from members of the aromatic series. In this connection we shall have occasion to refer to it again.

The first three tests described above (pp. 210, 211), when taken together, afford a means of discriminating between the different forms of albumin which are apt to occur in the urine.

(1.) If in a given case the tests 1–3 should all give positive results, it may be concluded that the proteid present is in the form of serum-albumin, usually in conjunction with a small proportion of globulin; but the presence or absence of peptone or albumose in addition remains undetermined.

(2.) If the urine contains but a small quantity of serum-albumin alone, only the first two tests will give any result.

(3.) If the first test fails to disclose the presence of albumin, while a precipitate is formed on the addition of acetic acid in the second, this may consist of mucin or resinous acids. In the latter case, it is soluble in alcohol.

(4.) If, on the application of test 1, no result is at first obtained, but a precipitate forms on cooling the specimen, this precipitate may be removed by filtration and tested by test 3 (biuret test), when the characteristic reaction will point to the presence of albumose. In such a case, the inference is strengthened if the pure or diluted urine responds to test 2, and if test 3, when applied to the urine directly, produces an abundant precipitate. Further investigation may then be conducted in the manner indicated at p. 221.

(5.) Again, if tests 1 and 2 are unattended with any result, if acetic acid alone will not cause a precipitate, and if test 3 at the same time discloses the presence of albumin, it may be concluded with certainty that the urine contains a large proportion of peptone. This condition is doubtless very rare. The author has met with it several times—repeatedly in the resolution stage of severe pneumonia, and further in a case of acute rheumatism, when serious and extensive joint symptoms were rapidly disappearing under the influence of preparations of salycylic acid. For the more accurate determination of peptone, it will usually be necessary to resort to the method described at p. 219.

By proceeding in the order indicated, it will be possible to form some judgment as to the character of the proteids under investigation, and this, as we shall see presently, is often a point of interest clinically.

(*β.*) **Quantitative Estimation of Albumin.**

1. *By weight.*—A certain quantity of the urine—60–100 cc., according as it is rich in albumin or otherwise—is placed in a beaker, and heated on the water-bath. A two per cent. solution of acetic acid is then added, drop by drop, until the albumin has separated in flocculent masses, when the fluid is boiled, and passed through an ash-free filter of known weight. The precipitate on the filter is washed successively with water, alcohol, and æther, and heated to a constant density at 120°–130° C. The filter is again weighed, and the difference in weight due to the precipitate is that of the albumin in the quantity of urine taken. To ensure greater accuracy in the result, it will be necessary to ascertain the amount of ash in the filter, and to allow for it. This may be accomplished by burning the filter with the albumin upon it in a platinum crucible of known weight.[117] For such determinations the use of glass wool filters is to be recommended.

2. *The Methods of Roberts, Stolnikow, and Brandberg*[118] for the approximate estimation of albumin are all based upon the principle of *Heller's* test, and are highly serviceable. The principle referred to may be expressed by the fact, that the richer the urine is in albumin, the more quickly turbidity is developed in the use of Heller's test. If in 100 cc. of urine there are 0.0034 grammes (*Roberts*), or 0.004 grammes (*Stolnikow*) of albumin, the fluid begins to cloud after the lapse of 35–40 seconds, and becomes plainly turbid in 1½ minute.

Brandberg's modification of Roberts' and Stolnikow's methods gives the best and most accurate results. This observer has found that in a solution of one part albumin in 30,000 of water, and consequently in urine holding 0.0033 per cent. of albumin, Heller's reaction will take place in 2½–3 minutes, and upon this fact he has based his method It is conducted thus:—The urine to be examined is first submitted directly to Heller's test. If a precipitate is obtained, a measured bulk

of the fluid is diluted in a graduated tube with nine times its quantity of water (1 in 10 urine), and Heller's test is again applied to the mixture in the following manner:—In a test-tube of about 1 c. diameter, some pure nitric acid is placed with a pipette, and in such a manner as not to touch the sides of the tube. The latter is now inclined, and the diluted (1 in 10) urine is carefully poured from a graduated burette along its lower surface, so that it may float upon the acid without blending. Two cc. may be placed in the test-tube in this way. Suppose now that an obvious clouding of the fluid takes place *before* the lapse of three minutes, the diluted (1 in 10) urine contains more than 0.0033 per cent., and the pure urine therefore more than 0.033 per cent. of albumin. If, on the other hand, the albuminous cloudy ring takes longer than three minutes to develop, it is known that the simple urine holds less than 0.033 per cent. albumin in solution. In the first case the mixture is further diluted by Brandberg in this manner:—Five test-tubes are taken, and in each he places 2 cc. of the diluted urine. To the first he then adds 4 cc. of water; to the second 13 cc.; to the third 28 cc.; to the fourth, 43 cc.; and to the fifth, 58 cc. He then repeats the test with each of these. If in the case of one of them the reaction occurs in the space of $2\frac{1}{2}$–3 minutes, it follows that the fluid in the particular test-tube contains 0.0033 per cent. albumin; and from this the proportion of that substance in the undiluted urine may be directly calculated by the following formula:

$$p = \frac{k + x}{k \,.\, 30}$$

Where

p = percentage of albumin in the undiluted urine.
k = the quantity of 1 in 10 diluted urine in each test-tube.
x = the quantity of water used for dilution.

Brandberg has compiled for the use of the practitioner a practical Table by which the percentage of albumin can be readily computed from these data without the trouble of calculation. It is reproduced here with slight modification. Its conclusions are based upon the supposition that turbidity ensues three minutes after the application of Heller's test:—

I. 2 cc. 1/10 urine,	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50 % albumin.
II.	1	4	7	10	13	16	19	22	25	28 cc. water.
I. 2 cc. 1/10 urine,	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00 % albumin.
II.	31	34	37	40	43	46	49	52	55	58 cc. water.
I. 2 cc. 1/10 urine,	1.05	1.10	1.15	1.20	1.25	1.30	1.35	1.40	1.45	1.50 % albumin.
II.	61	64	67	70	73	76	79	82	85	88 cc. water.

The figures in the horizontal line marked II. indicate the quantity of water in cc. which must be added to the 2 cc. of the 1 in 10 diluted

urine in order that turbidity may occur in three minutes. In line I. are exhibited the corresponding percentages of albumin in the specimen of urine taken.

In applying this method, it will be convenient to have a burette filled with the 1 in 10 diluted urine, and another containing distilled water. In each of one series of test-tubes may then be placed an equal quantity of nitric acid, with the precautions indicated above; and in those of another 2 cc. of the 1 in 10 diluted urine and varying quantities of distilled water (see Table). A preliminary experiment is first made, and according as this experiment shows an excess above or deficiency from the standard percentage of albumin, more or less diluted mixtures of 2 cc. 1/10 urine + water are prepared. These mixtures of urine and water in the second series are now tested by placing them with a pipette on the surface of the nitric acid in the test-tubes of the first series, until that one is reached in which the albuminous ring takes precisely three minutes to form, and from this the proportion of albumin in the urine is calculated. To take an example:—Suppose it is found in this way that turbidity occurs after the lapse of three minutes from the addition to the nitric acid of the fluid in a particular test-tube, and that this fluid has been formed by the admixture of 13 cc. of water to the 2 cc. of 1 in 10 diluted urine: reference to the Table will show that 0.25 corresponds to this number, 13, and consequently the urine contains 0.25 per cent. of albumin. If, however, the fluid in the test-tube to which 13 cc. of water has been added fails, when tested, to become turbid, or does so only after a longer interval, a less highly diluted mixture must be examined in a similar way—as, for instance, the contents of the tube to which 10 cc. water have been added, and so successively with the others (7 and 3 cc. dilution), until the required result is obtained. If, on the other hand, the mixture in the test-tube diluted with 13 cc. exhibits turbidity directly, the test must be repeated with those towards the other extremity of the scale—namely, those containing 16, 19, 22, &c., cc. respectively—until, as before, one is found in which the phenomenon occurs after the lapse of precisely three minutes. Let this be the fluid formed by the admixture of 25 cc. from the burette containing distilled water; then the Table will be found to give 0.45 as the corresponding figure, and the urine under examination, therefore, contains 0.45 per cent. albumin.

This method, when carefully conducted, is altogether reliable. *Hammarsten*[120] has compared its results with those obtained by the process of weighing, and has found that the two correspond within 0.206 per cent.

3. *Estimation of albumin by precipitation with picric acid and Esbach's albuminimeter.*[121]

The advantage of this proceeding is its simplicity. It is open to certain fallacies, contingent upon the unsatisfactory character of the reagent used (see p. 212); but it calls for description here as affording the physician a practical, if only approximate, means of determining the proportion of albumin in the urine.

Ten grms. of pure picric acid and 20 grms. of pure citric acid are dissolved in 900 cc. water. When the solution is thoroughly cool, water is further added to 1000 cc., and the mixture is employed as a precipitating agent. An instrument called the albuminimeter is further needed. This is a vessel resembling a test-tube, of stout glass. At its upper part it bears a mark, *R*, and lower down another mark, *U*. The lower third is graduated, and the divisions, which are numbered from $\frac{1}{2}$–7, are so disposed that the intervals between successive units diminish from below upwards (fig. 107). For the purpose of the experiment, the albuminimeter is used in the following manner:—It is filled with urine up to the mark *U*, and the reagent is then added until the surface of the fluid reaches to the mark *R*. The thumb is then applied to the mouth of the instrument, and it is reversed several times, until the fluids are well mixed. It is then covered with an india-rubber stopper, and allowed to stand upright for twenty-four hours. After this the height of the sediment in the tube is read off by the scale upon its surface. This is constructed so that the numbers express the proportion of albumin in grammes to the litre. Should it happen in a particular case that the upper limit of the sediment overtops the number 7 at the highest point of the scale, *i.e.*, where the proportion of albumin exceeds seven per cent., the experiment must be repeated with diluted urine. And it will save time if the urine be diluted at the outset, wherever it has been shown by a qualitative test to be comparatively rich in albumin. It occasionally happens that the albuminous precipitate will not cohere, or it may remain floating in the fluid. In such cases the method is inapplicable.

FIG. 107.
Esbach's Albuminimeter.

The quantity of albumin present can be estimated only approximately by this method. The experiments of *Czapek*[122] have shown that its merit is enhanced if certain precautions be observed in its use. The urine to be tested should be fresh and acid, and of low specific gravity, to which end it may be diluted. The fluid should not contain more than four grms. albumin to the litre, and should be kept for twenty-four hours in the albuminimeter at a moderate temperature

before the reading is taken. Finally, the figures on Esbach's instrument are too low. The best results are obtained where the urine is below 1.010 specific gravity, and does not hold more than 0.2 per cent. albumin. The conclusions set forth here are confirmed by *Sokolow*.[123]

This method is appropriate to the case of transitory or febrile albuminuria, and it is inapplicable where the urine may be supposed to contain quinine, antipyrin, or thallin.

From comparisons made by *Richter*, it appears that Esbach's method is far less accurate than *Brandberg's*. It is open to very considerable objections, and can be relied upon only within broad limits.

It may be noticed that *Huppert* and *Zadŏr*[124] have endeavoured to found a quantitative method of estimating albumin upon the comparative specific gravity of the urine. Its efficiency has not yet been sufficiently tested, but it may prove to be of service clinically.

2. Peptonuria.—This condition has been invested with very great interest since the discovery by *Hofmeister*[125] of a comparatively simple chemical test for the detection of peptone.

So far as our present knowledge extends, the causes of peptonuria are quite different from those to which the other forms of albuminuria are due. Neither nephritis, circulatory disturbances, nor anæmia, will bring about the appearance of peptone in the urine. Its presence there is most commonly—though not invariably—associated with such processes as are characterised by the collection and subsequent destruction of leucocytes under such circumstances that the products of disintegration, including the peptone constituent, of these bodies can obtain admission into the blood-stream, to be subsequently eliminated by the kidneys.

To the condition arising in this way the name *Pyogenic peptonuria* has been given (*Hofmeister*, *Maixner*, *v. Jaksch*).[126] It occurs chiefly in the resolution stage of pneumonia, in purulent pleuritic exudation, and, in general, in suppuration wherever situated, provided that the conditions are favourable to the absorption of the constituents (peptone) of the pus.

Peptone has further been found abundantly in the urine in purulent meningitis, acute articular rheumatism, the suppuration of phthisis—briefly, in nearly all those states which are attended with the formation and breaking down of pus.

It follows, therefore, that the detection of peptone in the urine goes far to warrant the inference that suppurative changes are in progress in some part of the system, but such an inference must, in all cases, be strengthened by the exclusion of the other known causes of peptonuria.

Amongst these are severe cases of scurvy, tending towards a fatal termination (*hæmatogenic peptonuria*) (*v. Jaksch*).[127]

Maixner [128] has also shown that in ulceration of the intestine, peptone derived from the food may pass directly into the blood through the ulcerated parts, and so give rise to this condition (*enterogenic peptonuria*), an observation confirmed indirectly by those of *Pacanowski.*[129] A number of cases have also been reported in which peptone found in the urine was attributed to phosphorus poisoning; and *Fischel* [130] has shown that it is normally a constituent of the urine in the puerperal state (*puerperal peptonuria*).

These facts are mentioned chiefly to show that peptonuria does not always imply suppuration; but these other conditions being excluded, it is a valuable diagnostic sign of that process. It is, moreover, available as a means of prognosis and of testing the progress of certain diseases attended with the resolution of pus. Thus, when peptone is found in the urine in the course of pneumonia, it indicates that the stage of softening has begun. Again, in connection with abdominal tumours or pleuritic effusion, it shows their purulent character; and in purulent meningitis, its manifestation varies with the severity of the disease—peptonuria occurring together with a relapse, and so on.

As a means of discriminating between tubercular and epidemic cerebro-spinal meningitis, the presence of peptone in the urine is occasionally a fact of crucial significance. It is characteristic of the latter disease, and its absence in presence of the clinical symptoms of meningitis in all cases implies a tubercular character. Obviously, however, peptonuria may arise accidentally in the course of tubercular meningitis; and in basing a diagnosis upon this condition, care must be taken to ascertain that it is not due to ulcerative processes in other organs, and especially to exclude implication of the lungs.

Again, in the condition which has been called "sepsis occulta," and which is commonly so difficult to recognise, peptonuria is an important symptom. By its aid especially it will be possible to distinguish the symptoms of septicæmia from those of latent disseminated sarcoma, which present quite a similar clinical character (high fever, rigors).

In a case which came under *Prof. Nothnagel's* care, there had been rigors and a high temperature maintained for a long period, and no other symptoms whatever. These were ascribed to the formation of a deep-seated abscess. The urine was repeatedly examined for peptone without result. The *post-mortem* showed disseminated sarcomatous nodules.

The author has been engaged for many years in investigating the subject of peptonuria, and he feels warranted in asserting that its clinical value as a symptom is very great. It may be that further experience will extend and distribute its import; but there can be little doubt that the significance attaching at present to the form with which we are best acquainted—that of *pyogenic peptonuria*—will not diminish

with the progress of knowledge. Recent investigations tend in the fullest manner to confirm this view.[131] Peptonuria is frequently met with in connection with syphilis (*Poehl*).[132] Modern science furnishes an adequate explanation in many instances where the condition is found, for granting that micro-organisms have the property of changing albumin into peptone, it is probable that they can also cause the latter to appear in the urine (*Mya*, *Belfanti*).[133]

Estimation of Peptone.—Hofmeister's[134] method is the best for this purpose. Many modifications have been introduced in recent years, but none seem to possess any advantage over the original, and some are subject to fallacies which do not belong to it. In examining for peptone, the following procedure may be adopted :—

The urine should first be tested for albumin by the three processes described above (pp. 210, 211). If tests 1 and 2 give no result, and no precipitate forms on the addition of acetic acid alone, the presence of peptone may be shown by the biuret test (see p. 211), but only when this body is in great abundance. If it is not so, the result in this case will be also negative. A further preliminary test may be applied by adding first concentrated acetic acid, and then a mixture of acetic and phospho-tungstic acids. If clouding takes place either directly or after the lapse of a short interval, it may be concluded that peptone is present. The inference is rendered still more certain if, before the application of the test, a little neutral acetate of lead be added (to precipitate mucin), until a flocculent precipitate appears. If the test again gives positive results, peptone is present; if it remains negative, the urine is peptone-free. This, however, only holds good where the urine contains a considerable amount of peptone.

Hofmeister's test is, as mentioned, more accurate. Assuming that the previous methods have failed to disclose albumin, the urine is treated with neutral acetate of lead and filtered. The clear filtrate, which should amount to not less than 500 to 600 cc. in volume, is acidulated with hydrochloric acid, and phospho-tungstic acid is added until a precipitate ceases to form with it. The fluid is then filtered without delay.

Phospho-tungstic acid may be prepared thus :—Commercial tungstate of soda is dissolved in boiling water and phosphoric acid added until the mixture exhibits an acid reaction. It is then allowed to cool, rendered strongly acid with hydrochloric acid, and filtered after standing for twenty-four hours (*Huppert*).[135]

The precipitate consists of peptone combined with phospho-tungstic acid, and various other substances (animal alkaloids, &c.). It is now washed on the filter with five parts concentrated sulphuric acid in 100 of water, until the fluid which passes through is colourless. In this way the salts are got rid of. The precipitate, while still wet, is washed

from the filter with as little water as possible, and is received in a watch-glass. Barium carbonate is added until the mixture is alkaline, and the latter is then placed on a water-bath at boiling-point, and heated for ten to fifteen minutes, and the biuret test (p. 211) applied. Peptone is shown by the formation of a colour ranging from bluish-red to violet, and varying in intensity according to the quantity present. If this be only a trace, the resulting colour is of a dirty red or dull violet. The accompanying precipitate of baryta need not confuse the experiment; but should any doubt exist as to its result, the entire preparation may be placed in a test-tube and allowed to stand for a few minutes. The precipitate then falls to the bottom, and the fluid displays the characteristic tints—from dull red to violet—if it contains peptone. If that body be absent, it has a greenish colour.

If either of the first two tests (p. 210) should show the presence of albumin, whilst the addition of acetic acid and ferrocyanide of potassium after filtration causes only *very slight* turbidity, the albumin must be removed by combination with a metallic oxide—and best with oxide of iron—in the following manner:—A solution, first of acetate of soda and then of perchloride of iron, is added to the urine. This is exactly neutralised with caustic potash, boiled, filtered, and allowed to cool. Tests 1 and 2 are next applied. If neither discloses albumin, and if also with test 2 no blue colour forms, thus showing that the fluid is free from iron, the further process is that described above—hydrochloric acid is added, and a precipitate obtained by the use of phosphotungstic acid, and so on, as before.

If, however, after the precipitation of albumin in the manner indicated, one of the tests alluded to should show that the fluid is not yet free from that body, the previous tests must be repeated until the filtrate exhibits no trace either of albumin or of iron. Should the former substance be present in the urine in great quantity, it may be removed in large proportion by heat, and what remains may be subsequently got rid of in the manner described above.

The application of this method in the case of highly coloured albumin-free urine has the contingent advantage that it involves the removal of colouring matters whose presence might be a source of fallacy. *J. A. Schulter*[136] recommends that the urine be first saturated with ammonium sulphate, and the filtrate proceeded with as before. The quantitative estimation of peptone may be effected by the colorimetric process of *Hofmeister* and *Maixner*.[137] [See Appendix XIX.]

3. Albumosuria.—The distinctive character of this condition was formerly thought to be the presence in the urine of a single body, to which the name of propeptone or hemialbumose had been given. The researches of modern times, however (*Kühne*, *Chittenden*, *Herth*[138]),

have placed the matter in a new light, and the first two observers have sought to show that propeptone is really a mixture of four different proteids. However this may be, these interesting observations cannot as yet be brought to bear upon our clinical knowledge.

Albumose has been found in the urine in connection with various diseases—as osteomalacia, dermatitis, intestinal ulcer, &c. (*Senator*, *Ter Gregoriantz*, *v. Jaksch*).[139] In two severe cases of osteomalacia, which were recently under treatment by Professor Nothnagel, the author failed to discover albumosuria.

The clinical significance of this condition, indeed, is in general somewhat indefinite, and it does not lend itself very surely to the aid of diagnosis. *Loeb*[140] believes that he has discovered propeptone in the urine in measles and scarlatina, and from the observations of *Kahler* and *Huppert*[141] albumosuria would seem to be a frequent occurrence in inflammation of the medulla of bones. The clinical significance of this state is modified by the fact that *Posner*[142] has detected propeptone in the seminal fluid (see Chapter IX.). The presence of albumose, when it occurs alone, may be determined by the consideration of results derived from the tests given for the detection of albumin generally. If, on the application of tests, a precipitate first forms on cooling the specimen, or when it has been allowed to stand for a long time (*vide supra*), and if this precipitate, when separated on the filter, is shown by the biuret test to consist of albumin, a fresh specimen of the urine may be submitted to test 2. In doing this, it may be necessary to add water, since albumose is readily soluble in concentrated salt solutions, and therefore in concentrated urine. Whether with or without this precaution, a further precipitate with test 2 suggests the presence of albumose. Another specimen should now be saturated with common salt, and further treated with acetic acid. If albumose be present, a precipitate forms, which, after the addition of a large amount of acetic acid, dissolves when heated and reappears on cooling. A nitrogenous body resembling albumose was found by *Thormählen*[143] in the urine in a case of hydatids of the liver, with jaundice and nephritis.

When the urine holds albumose in conjunction with serum-albumin, the latter must be removed by boiling with acetic acid and chloride of sodium before the tests are applied.[144]

4. Globulinuria.—Globulin probably never, or almost never, occurs alone in the urine, but generally in conjunction with serum-albumin. Consequently its import in disease is the same as that of the latter body.

To *Kauder*[145] belongs the credit of discovering a simple method for the detection of this body in presence of serum-albumin. The urine is rendered alkaline with ammonia, allowed to stand for an hour, then

filtered, and to the filtrate is added its own volume of a saturated solution of sulphate of ammonium. If globulin be present in any quantity, a flocculent precipitate falls. [See Appendix XX.]

By an extension of this method the quantitative estimation of globulin may be effected. With this object the precipitate formed is treated in the manner described before when speaking of the estimation of albumin by weight (p. 213), (*Pohl*[146]).

5. Fibrinuria.—Fibrin is found in the urine in cases of hæmaturia and chyluria (*v. infra*). It then usually forms coagula. It is found, moreover, as a consequence of inflammatory exudation in the urinary passages. The coagula occur most commonly in croup and diphtheria, and occasionally in tuberculosis.

To detect fibrin the clots should be separated by filtration, washed with water, dissolved by boiling in solution (1 per cent.) of soda or (5 per cent.) hydrochloric acid (*Huppert*[147]), and the fluid tested when cold by the process described on p. 210.

6. Hæmaturia.—The blood which occurs in the urine may be derived from several sources, from the kidney, renal pelvis, ureters, bladder, or urethra (see p. 176).

In well-marked cases, the presence of blood is directly suggested by the colour of the urine, which varies from that of an extract of raw meat to a ruby-red; but inasmuch as a similar appearance may be due to hæmoglobin in solution (hæmoglobinuria), it is never safe to base the diagnosis of this condition upon inspection alone. It may be accurately determined by—

1. *Spectroscopic Examination.*—The freshly-passed urine, which, if deeply coloured, should be diluted with water, will exhibit the two absorption-bands of oxyhæmoglobin (p. 38, fig. 22), and these, on the addition of sulphide of ammonium, give place to the absorption-band of reduced hæmoglobin. Lastly, when blood containing urine has stood for some time, occasionally even in fresh urine, the spectrum of methæmoglobin may be looked for (p. 40, fig. 28).

2. *Heller's Test.*[148]—The urine is treated with caustic potash and boiled. The (basic) earthy phosphates are then precipitated, and together with them the hæmatin derived from the oxyhæmoglobin present. The phosphatic sediment is consequently coloured a bright red. Should it happen that the urine contains abundance of colouring matter (bile-pigment, &c.), which renders it difficult to appreciate the colour of the sediment, the latter should be separated on a filter and dissolved in acetic acid. The solution then becomes red if blood be present, and its colour vanishes gradually on exposure to the air.[149]

Rosenthal[150] applies the test for hæmin directly to the dried precipitate (see p. 39). *Struve's*[151] method serves well for the detection

of blood-pigment. The urine is treated with ammonia or caustic potash, and the fluid then rendered acid with tannic and acetic acids. The presence of blood is shown by a dark-coloured precipitate, which, when dried and treated with a little chloride of ammonium and glacial acetic acid, should yield *Teichmann's* crystals. This test is very sensitive, but less practicable than Heller's, which, with the spectroscopic examination, best meets the requirements of the physician.

If, in addition, the microscope reveals the presence of red blood-corpuscles (p. 175), the existence of hæmaturia may be inferred, and it only remains to judge of its origin on the principles already laid down. For its clinical significance the reader may refer to p. 176.

7. Hæmoglobinuria.—Sometimes the colouring-matter of the blood is also found dissolved in the urine (see p. 43). This condition is apt to arise in the course of acute infectious diseases, in burns, and in various forms of poisoning. In the latter class of cases it is always a serious, and even ominous symptom. It has been observed in poisoning by naphthol [152] and carbolic acid.[153] Hæmoglobinuria is known as an idiopathic affection [154] (paroxysmal hæmoglobinuria), and as such often occurs in connection with severe syphilis.

The occurrence of hæmoglobinuria may be inferred when it is made evident by spectroscopic analysis and the application of Heller's test that the urine contains blood colouring-matter, whilst at the same time the microscope discloses smaller or larger masses of brown pigment, and either no red corpuscles, or so few as are inadequate to account for the results obtained. The spectroscopic appearances are usually those of methæmoglobin (p. 40, fig. 28); and *Hoppe-Seyler* [155] maintains that in this condition the blood-pigment is always in the form of methæmoglobin.[156]

8. Mucinuria.—The presence of small quantities of mucin in the urine is not a pathological symptom; when found in greatly increased proportion in women, it is often derived from the vagina. Such an increase, originating in the urinary passages, invariably points to catarrh of those parts. The urine in question is usually turbid when passed, and after a little while it deposits a bulky cloud. The latter is seen by the microscope to contain leucocytes and epithelium (p. 176). When a great quantity of mucin is passed, it forms a viscid gelatinous sediment at the bottom of the urine-glass, and no further evidence of its character is needed.

For the detection of mucin chemically, the urine is treated with an excess of acetic acid, when it is rendered turbid if much mucin is present. It may be necessary to dilute it previously, since concentrated urine, being rich in salts, will retain mucin in solution even in presence of acetic acid. In testing for the presence of mucin in albuminous

urine, the great bulk of the albumin should be removed by boiling and filtering previously, and the filtrate allowed to cool.

The best method for precipitating mucin from urine is by the addition of acetate of lead (*vide supra*).

II. Carbohydrates.

1. **Glycosuria.**—Although various forms of sugar are occasional constituents of the urine, as, *e.g.*, sugar of milk after parturition, and inosite and lævulose rarely, the consideration of these will nevertheless hardly detain us, since their manifestation, from the point of view of frequency and importance, possesses but little practical interest in comparison with that of grape-sugar (glucose, dextrose). Our remarks here will be confined to the occurrence and tests for the latter body.

(*a.*) **Physiological Glycosuria.**—It must be mentioned at the outset that normal urine contains a trace of sugar. The fact of a physiological glycosuria was long ago laid down by *v. Brücke*,[157] and has quite recently derived remarkable confirmation. *Wedenski* [158] has made use of *Baumann's* discovery that benzoyl chloride forms insoluble compounds with the carbohydrates; and applying this to healthy urine, he succeeded in separating from the precipitate a body which gave all the reactions of grape-sugar. The proportion to be found in health, however, is so small, that it may be neglected as a disturbing factor, even in the most sensitive of the tests to be described.

(*b.*) **Pathological Glycosuria.**

(*α.*) *Transitory Glycosuria.*—Grape-sugar may appear temporarily in the urine in the course of many diseases, as cholera, intermittent fever, cerebro-spinal meningitis, and scarlatina,[159] in affections of the brain involving the fourth ventricle, in diseases of the heart, liver, and lungs, and in gout. It has also been occasionally observed in small quantities in cirrhosis of the liver. Glycosuria in connection with these diseases is, however, very rare. It is commoner as an effect of certain poisons, notably morphia and carbon monoxide. The author has found grape-sugar in the urine in two cases of advanced asphyxia from breathing irrespirable gases (a mixture of carbonic acid and nitrogen).

(*β.*) *Persistent Glycosuria.*—The continued excretion of grape-sugar in appreciable quantities belongs exclusively to diabetes mellitus, and it is the most certain symptom of that disease. Its great clinical importance lies in the fact that it ordinarily becomes apparent at a time when all other symptoms of diabetes are wanting. In such cases, however, one can be certain that diabetes exists only when, by repeated investigation, grape-sugar is found, and especially when its quantity is observed to increase with the administration of other carbohydrates, as cane-sugar,[160] and still better, starch.

Determination of Grape-Sugar.

(*a.*) **Qualitative Tests.**—It is very easy to determine the presence of a considerable proportion of sugar in the urine; but sometimes, when that body occurs only in traces or in very small quantity, the tests hitherto most commonly employed, namely, those of *Moore* and *Trommer*, are hardly sufficient for its detection. We have only recently become acquainted with a method which is in all cases adequate to the purpose.

1. *Moore-Heller Test.*[161]—The urine is treated with liquor potassæ and boiled. If sugar be present, it is decomposed. Lactic acid and a number of volatile compounds are formed,[162] and with them certain coloured substances, which impart an intense deep-brown tint to the fluid. This test is by no means accurate, and conclusions drawn from it are open to fallacy, since healthy urine turns brown with caustic potash from the action of that body upon mucin. Moreover, the change of colour is proportional to the quantity of mucin present, independently of sugar.

2. *Trommer's Test.*[163]—The urine is rendered alkaline with caustic potash, and a fairly-strong solution of cupric sulphate is added, drop by drop, until the cupric oxide formed ceases to be dissolved. The mixture is then heated in a test-tube. If sugar be present in greater quantity than a mere trace, a yellowish or red precipitate of the suboxide of copper falls before the boiling-point is reached, and at the same time the fluid loses colour somewhat. This test is very sensitive. Trommer was able with it to detect sugar to the amount of 0.001, or even 0.0001 per cent. Unfortunately it is also ambiguous. The property of reducing cupric oxide in alkaline solutions belongs to a number of bodies which occur in healthy and morbid urine. Amongst these are uric acid, kreatin and kreatinin, allantoin, mucin, milk-sugar, pyrokatechin, hydrochinon, and bile-pigments. In addition to these, the ingestion of benzoic and salicylic acids, glycerine, and chloral leads to the formation in the system of substances which possess a similar reducing power. Hence it happens that the urine is sometimes thought to contain sugar on the evidence of Trommer's test when none can be found with other methods. The error is especially apt to arise when the boiling is continued for a long time. *The test can be depended on, therefore, only when reduction takes place at a temperature below boiling, which, however, occurs only when the urine contains a relatively large proportion of sugar.*

Fehling's fluid (p. 231) may be substituted for the copper sulphate and caustic potash in the process.

A useful modification of Trommer's test has been suggested by

Worm-Müller.[164] A mixture is made of 1.5 cc. of a 2.5 per cent. solution of cupric sulphate, and 2.5 cc. of an alkaline solution of tartarated soda and potash (prepared by dissolving 100 grms. of Rochelle salts in a normal solution of caustic soda), and heated to boiling-point; and in a separate test-tube 5 cc. of the urine to be tested are boiled. The boiling fluids are added together without shaking them, and if sugar be present in any quantity, the suboxide is precipitated directly. Should no such precipitate form, the process is repeated with 2, 3, or 4 cc. of the cupric sulphate solution. This test is said to be very sensitive.

It should be mentioned that the power of a urine to dissolve cupric oxide does not necessarily imply the presence of sugar in the urine, since the property belongs to ammoniacal and albuminous urine, whether it contains sugar or not.

3. *Fermentation Test.*—This depends upon the fact that grape-sugar decomposes in presence of yeast into alcohol, carbonic acid, and a number of other products (succinic acid, glycerine). It is conducted in the following manner:—A test-tube is filled for two-thirds of its depth with mercury, and in the remaining third with the urine, to which a little tartaric acid has been added. In this is placed some yeast which has been carefully washed. The mouth of the test-tube is then closed with the thumb, and it is inverted over a vessel containing mercury. If sugar be present, fermentation takes place directly, and the carbonic acid formed collects over the mercury. There are special fermentation tubes made for such purposes, and the experiment is greatly facilitated by their use.[165] This test is sufficiently sensitive. It will serve to indicate 0.1 per cent. of sugar in the urine.[166]

4. *Phenyl-hydrazin Test.*—In this we have a method for the detection of sugar which is greatly to be preferred to those already mentioned. Several years' experience has convinced the author that it is entirely reliable, simple in its application, and in every way suited to the needs of the physician. It depends upon the property of phenyl-hydrazin to form with grape-sugar a highly-characteristic crystalline compound[167] called phenyl-glucosazon. This body has the form of yellow needles, and is but little soluble in water. The test is conducted in the following manner (*v. Jaksch*[168]):—Two parts of hydrochlorate of phenyl-hydrazin * and three of acetate of soda are placed together in a test-tube containing 6–8 cc. of urine. If the salts do not dissolve when the fluid is warmed, a little water is added, and the test-tube containing the mixture is placed for 20–30 minutes in boiling water. After this it is taken out and put into a vessel

* Twice as much of the phenyl-hydrazin salt as will lie on the point of the blade of a knife.

containing cold water. If sugar be present, even in very moderate quantity, there forms directly a yellow crystalline deposit, which may appear amorphous to the naked eye, but which when examined under the microscope is seen to contain yellow needles detached or arranged in clusters (fig. 108). If the urine holds but a very small proportion of sugar, the preparation should be placed in a conical glass, and the sediment examined carefully. In a case where only a mere trace of sugar exists, detached crystals of phenyl-glucosazon cannot fail to be seen. The discovery of smaller and larger yellow scales, or of powerfully-refracting brown granules, must not, however, be mistaken for evidence of sugar. This test gives *very reliable* results with every variety of morbid urine, and it is equally applicable whether albumin be present or not. In the former case, however, it is well to get rid of the excess of albumin previously by boiling. Phenyl-glucosazon

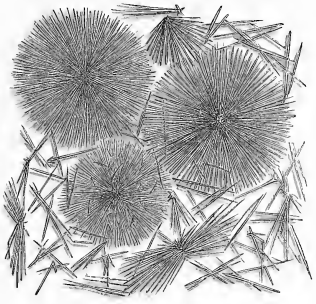


Fig. 108.—Phenyl-glucosazon Crystals from Urine containing Sugar.

crystals melt at 205°, and their character may be ascertained beyond doubt by submitting them to that temperature. The efficiency and utility of this test is amply proved.[169]

In addition to the foregoing methods, which especially merit attention, other tests for sugar have been suggested, and some of these call for description.

5. *Böttger's Test.*[170]—A quantity of the urine is mixed with its own bulk of a concentrated solution of carbonate of soda, and a little basic nitrate of bismuth is added. The preparation is then boiled. If sugar be present, it turns black from the reduction of oxide of bismuth. This process has no advantage which does not belong to Trommer's test, and it is less sensitive. If the urine contains the principles of rhubarb taken as food, a black precipitate will fall independently of sugar.[171] In albuminous urine, too, sulphide of bismuth will

form a similar black deposit.[172] These conditions must therefore be excluded.

The modification of *Böttger's* test by *Nylander*[173] is more accurate and reliable. The reagent employed (called *Almén's* fluid) is prepared by dissolving 4 grms. of Rochelle salts (tartarated soda and potash) in 100 grms. of an 8 per cent. solution of caustic soda, warming the fluid, and adding as much basic pernitrate of bismuth as will remain in solution. The mixture so formed is added to the urine to be tested in the proportion of 1 in 11, and the whole is then heated. The fluid should blacken in the course of a few minutes. In this way it is asserted[174] that sugar in the proportion of 0.1 per cent. can be detected. It has, moreover, the advantage of simplicity, but is also open to certain fallacies. It is inapplicable, as we have seen, to the case of albuminous urine, and the reaction occurs in presence of melanin or melanogen, or where the fluid contains a large proportion of reducing substances but no sugar.

6. *Mulder's Test.*—The urine is treated with carbonate of soda in solution, and solution of indigo-carmine is added until the whole is freely coloured. If sugar be present, the colour changes to yellow on heating, and becomes again blue when the fluid is shaken up with air.

This test may be conveniently applied thus:[175]—Two pieces of filter-paper are taken. One is placed in a concentrated solution of carbonate of soda, and the other in a solution of indigo-carmine. Both are then dried. When wanted for use, a small piece of the indigo-carmine paper is placed in about 10 cc. of water, the urine under examination is added, and finally a large slip of the paper saturated with carbonate of soda is placed in the fluid. The result should be as above. The convenience of its application in this manner is the only merit of Mulder's test. It is neither sensitive nor accurate.

7. *Johnson's Test* (*Picric Acid*).—Both *Johnson* and *Thiéry*[176] have employed picric acid as a test for sugar. A few drops of a solution of picric acid are added to the urine, which is then treated with caustic potash. If sugar be present, the fluid assumes a deep-red colour; but a red colour may also be obtained from caustic potash and picric acid alone, or in presence of kreatinin; and, consequently, as a test for sugar, the process is not reliable.

8. *Penzoldt's Test.*[177]—In this the reagent is diazobenzol-sulphonic acid dissolved in water, 1 : 60, without the aid of heat, but a drop of caustic potash may be added to facilitate solution. A few cubic centimetres of the urine under examination is placed in a test-glass, and rendered strongly alkaline with caustic potash. A like quantity of the reagent, which should be feebly alkaline, is now added. The pro-

cess is repeated with healthy urine of the same tint and concentration, and the two specimens compared. Both are at first of a reddish-yellow colour, but whilst the healthy specimen remains unchanged, or nearly so, that containing sugar becomes a bright claret-colour after some time. If sugar be present in abundance, the fluid eventually becomes dark-red and opaque.

Penzoldt asserts that 0.1 per cent. sugar is appreciable in this way; but for practical purposes the test is not to be recommended, since acetone and diacetic acid have a similar reaction.[178] Moreover, the substance used is highly explosive.[179]

9. *Rubner's Test.*[180]—The urine is treated with an excess of acetate of lead (sugar of lead) filtered, and to the filtrate ammonia is added until a permanent precipitate forms. The fluid is then heated, but not boiled. If sugar be present, the precipitate formed on the addition of ammonia gradually assumes a rose-red colour, which vanishes slowly on standing, more quickly on the application of heat (60°–70° C.), giving place to a yellowish-coffee colour.

Rubner believes that the precipitate consists of sugar of lead. Milk-sugar does not give this reaction when the process is conducted as above, but when a solution of that body is boiled for 3–4 minutes with acetate of lead, and ammonia then added, a similar precipitate forms. In the author's experience, the best plan in performing the test is to heat the precipitate gradually at a temperature not exceeding 80° C. By its use Penzoldt has discovered sugar to the amount of 0.01–0.02 grms. in 10 cc. of urine. That writer employs a very simple and practical modification of Rubner's test.[181] He adds to the urine a few drops of basic acetate of lead (subacetate) and a few of ammonia, and then warms the mixture. The presence of sugar causes a red precipitate as before. This method is not less sensitive than the other.

10. *Molisch's Reactions.*—*Molisch* [182] has recently devised two methods, by the aid of which he believes that sugar can be detected in the urine, whether of health or disease.

(*a.*) The first depends upon the reaction of sugar with α-naphthol and sulphuric acid. To obtain this, he takes ½ to 1 cc. of the fluid containing sugar—urine should be highly diluted for the purpose—places it in a test-tube, and adds to it two drops of a 15–20 per cent. alcoholic solution of α-naphthol. The fluid becomes turbid from the precipitation of some of the α-naphthol. Concentrated sulphuric acid is now added in excess, and the whole is well mixed. The presence of sugar is shown by the transitory appearance of a blue colour, and the formation of a violet-blue precipitate on the subsequent addition of water.

(*b.*) This reaction is obtained with thymol and sulphuric acid. The urine which is thought to contain sugar is highly diluted, and to ½ 1

cc. in a test-tube is added first two drops of a 15–20 per cent. alcoholic solution of thymol, and then sulphuric acid in excess. When the mixture is shaken, the momentary development of a "cinnabar-ruby-carmine red" discloses the presence of sugar, this colour giving place to carmine when the fluid is diluted with water.

By this method Molisch maintains that so small a proportion as 0.00001 per cent. of sugar can be detected. Similar reactions, however, may be obtained with cane-sugar, fruit-sugar, and maltose. *Seegen*[183] has further investigated the subject, and found that chemically-pure solutions of proteids, and especially of serum-albumin, behave in like manner. The author has repeatedly performed the test with albuminous urine, with the result that the α-naphthol reaction was obtained when the fluid was diluted in a much higher degree than that (1 : 100) recommended by Molisch in the case of sugar. In presence of albumin, the dark-violet colouration of the fluid was followed by the deposition of a greenish-black precipitate. The thymol and sulphuric acid reaction with this body was almost identical with that displayed by sugar.

For these reasons, Molisch's reactions, which no doubt find a valuable application in vegetable physiology, are not to be recommended as a test of glycosuria. The researches of *Mylius* and *v. Udransky*[184] have made it clear that Molisch's reaction is identical with the furfurol reaction, which takes place not only with sugar, but with any carbohydrate. It remains to mention that experiments have been instituted by several investigators (*v. Brücke*, *Seegen*, *Abeles*, *Salkowski*[185]) with the object of separating the sugar when it exists in very small quantities in the urine, and submitting it in the concentrated form to the action of Trommer's and other tests.

The separation and detection of grape-sugar, as of the carbohydrates generally, may be effected by the action of benzoyl chloride, which with the carbohydrates forms insoluble benzoic acid æthers. For this purpose the urine is treated with benzoyl chloride and caustic potash. A precipitate results. The addition of concentrated sulphuric acid and a few drops of an alcoholic solution of α-naphthol, causes an intense red colour if a trace of a benzoyl combination with the carbohydrate be present, and the coloured fluid will exhibit a well-defined absorption-band in the green part of the spectrum.[186]

(β.) **Quantitative Estimation of Grape-Sugar.**

1. *Titration Method* (*Fehling*[187]).—The principle upon which this method is founded depends upon the property which grape-sugar possesses of reducing cupric oxide to its suboxide in alkaline solutions. It has been applied in various ways, and most of these are to be found described in the text-books of urinary chemistry. Fehling's process has hitherto been chiefly in vogue, but, in common with most of the others,

it is tedious and difficult, and consequently but little suited to the requirements of the physician. At once the simplest and most satisfactory mode of procedure is that adopted by *Leube* and *Salkowski*.[188]

An opinion is first formed from the density of the urine as to the quantity of sugar which it probably contains. It is then diluted to such an extent that this shall not exceed 0.5 per cent. of the entire fluid—6–10 times its volume of water may be needed for the purpose.[189] The diluted urine is placed in a burette. Ten cc. of Fehling's solution are measured into a white porcelain capsule, 40 cc. of water added, and the bluish mixture heated until it begins to boil. The diluted urine is now slowly added, and a precipitate of suboxide (red) or its hydrate (yellow) falls, whilst the fluid loses its blue colour. It is important to ascertain the precise point at which this colour has disappeared entirely, showing that the fluid no longer contains free sugar. When this is reached, 1 cc. of the fluid is taken and filtered through thick filter-paper. The filtrate, which should be quite clear, is acidified with acetic acid, and a little ferrocyanide of potassium is added. The presence of copper will be shown by a brown colour, and 0.5–1 cc. of the diluted urine must then be added until the brown colouration no longer takes place. On the other hand, should it happen that the fluid when tested is seen to be free from copper, the whole process must be repeated with a smaller quantity of urine. Finally, when, as sometimes happens, suboxide of copper is not precipitated, and passes through with the filtrate, the experiment is entirely frustrated. When these points have been attended to, the process is repeated, that quantity of urine being taken at the outset which was found to be requisite. A calculation is then made by multiplying the number which expresses the dilution (in volumes) of the urine by 5 (10 cc. of Fehling's fluid correspond to 0.05 grm. sugar), and dividing the product by the number of cc. of diluted urine employed. The result is the percentage of sugar in the original urine. *Salkowski*[190] has suggested that the cuprous oxide formed should also be weighed. And *Munk*[191] recommends that a few drops of a 15.8 per cent. solution of chloride of calcium be added to the mixture of urine and Fehling's solution, to promote the precipitation of the copper salt.

Fehling's fluid is prepared as follows:—(1.) 34.639 grms. of pure crystalline cuprous sulphate are weighed out and dissolved in water with the aid of a little heat, water is then added to 500 cc., and the whole placed in a tightly-stoppered flask. (2.) 175 grms. of Rochelle salts (potassium and sodium tartrate) and 100 cc. of caustic soda of 1.34 sp. gr. are dissolved in 500 cc. of water, and well mixed, and also placed in a well-stoppered bottle.

When required for use, equal parts of these solutions are measured with a pipette, and the mixture constitutes Fehling's fluid.

[*Mr. Martindale* recommends the following formula:—(1.) Sulphate of copper,

181 grains; distilled water to 6 ounces. Dissolve. (2.) Tartrate of potassium, neutral, 728 grains; caustic soda, 360 grains; distilled water to 6 ounces. Dissolve.]

Of a mixture of these two solutions (Fehling's fluid) in equal volumes, 10 cc. will be decolorised and reduced by 0.05 grm. of glucose or diabetic sugar in solution.

2. *By Fermentation.*—This method of analysis was first suggested by *Roberts*,[192] and *Worm-Müller*[193] applied it to determine the proportion of sugar in the urine from the density of that fluid before and after fermentation. According to *Worm-Müller*, this method, with the aid of a thermometer and a pycnometer fitted with a graduated index-scale, gives reliable results where so little as 0.5–1 per cent. of sugar is present. Roberts concluded from experiments that a difference of 0.001 sp. gr. corresponded to 0.23 per cent. of sugar, and arrived at the following formula:—

$$x = \frac{D \times 0.230}{0.001}$$

Where

x = the percentage of sugar.

D = the difference in the density of the urine before and after fermentation.

It is possible to obtain good approximate results from the application of this method clinically. The following apparatus is required: two hydrometers accurately graduated to four places of decimals, and each provided with a thermometer carrying a fractional index, and capable of registering $\frac{1}{10}^{\circ}$ Celsius. These instruments should measure densities ranging between 1.000–1.025 and 1.025–1.050 respectively up to four places of decimals.

The first step is to take the sp. gr. of the urine at the temperature for which the hydrometer in use was constructed. This may be done by placing the test-glass containing it in a vessel of water, which may be cooled or heated as required. 100–200 cc. of the urine are then placed in a flask, together with some fresh yeast which has been carefully washed on an ash-free filter to secure removal of inorganic impurities. The flask is next closed with the arrangement represented in fig. 109, by which evaporation is prevented. Fermentation is allowed to go on for 24–48 hours. After this, the fluid, which should be clear, or nearly so, is passed rapidly through a filter, and its density again taken at the appropriate temperature, the latter being secured and ascertained as before. From the observations made, the percentage of sugar is calculated by the formula given above. The result is fairly approximate. [See Appendix XXI.]

3. *By Polarisation.*—Grape-sugar possesses a dextro-rotatory power for polarised light, and upon this fact a quantitative test for that body is based. Such a test must necessarily be open to fallacy, inasmuch as

the urine in diabetes is apt to contain other substances, such as β-oxybutyric acid and lævulose, which rotate light in the opposite direction. It is well, therefore, in applying it, to do so both before and after fermentation (*Hoppe-Seyler, Külz, Worm-Müller*), when the difference in the results will be a measure of the quantity of grape-sugar in solution. The advantage of the method is that it dispenses with delay. In recent times it has been invested with great accuracy by the use of a

Fig. 109.—Flask for the Approximate Estimation of Sugar by Fermentation.

polarimeter constructed on the principle devised by *Lippich*. This instrument is represented in fig. 110.[194] It is employed thus:—

The graduated disc is turned towards the observer and the tube towards the lamp, and the cap which covers the telescope and the posterior end of the apparatus is removed. The lamp is placed at a distance (45 cm.) equal to the length of the instrument itself. Carbonate of soda is placed in the cage attached to the lamp, and fused until the receptacle is full. The latter is then fixed in the lamp in such a way as to be in contact with the flame at the side only, and a screen is so placed that the light passes only through the aperture in it.

The following description will sufficiently explain the construction of the instrument:—At the hinder end of the polarimeter is a rod supporting the arc of a circle, and behind this, in the direction of the lamp, another rod, with a notch marked upon it. The second rod is fitted with a screw, by means of which it moves upon the first, and can be fixed in contact with the latter. When the line upon the upper surface of the adjustable rod corresponds to the central point (0) of the metallic arc, and this with the 0 of the vernier, the entire field is dark, and by rotating the arc (which is done with the ivory lever in front) both halves will be obscured or illuminated equally. In experi-

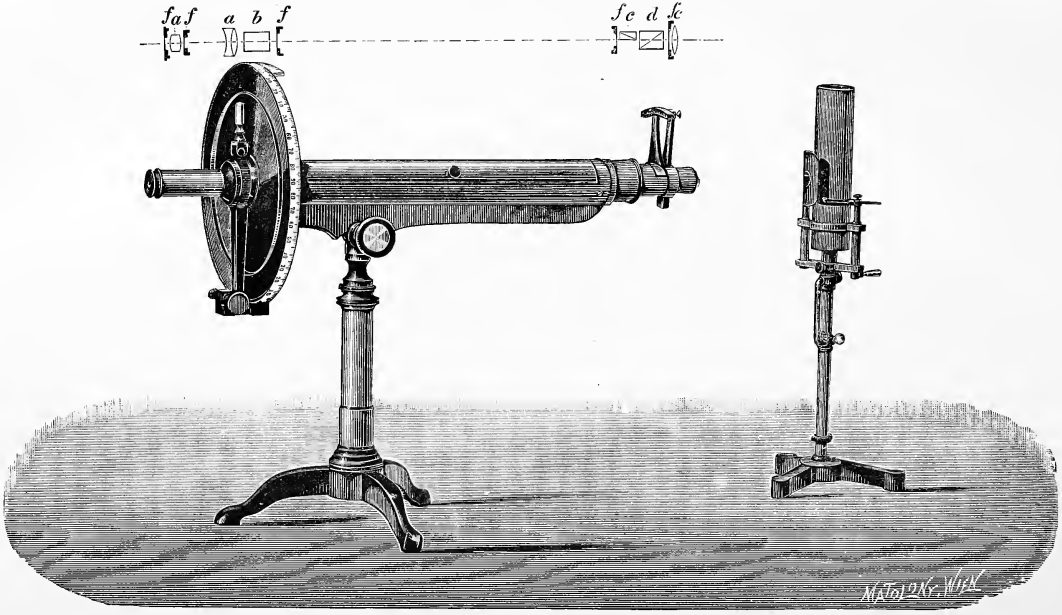


FIG. 110.—Lippich's Polarimeter.

a. Astronomical telescope.
b. Analyser (Nicol).
c. Half-prism (Nicol) fixed (polariser).
d. Complete prism (Nicol) movable (polariser).
e. Illuminating lens.
f. Diaphragms.

The diagram shows a horizontal section of the instrument as seen from above.

menting with the instrument, the adjustable rod is deflected slightly to the right or to the left. It is practically a lever attached to the box containing a Nicol prism (*d* in the section, which is represented as seen from above), and rotates the prism on its axis.

In conducting an observation, the tube containing the urine or other fluid to be examined is placed in the capsule, and the second rod is moved right or left, say as far as the mark 4. The observer then looks through the telescope, and adjusts the instrument in such a manner that the field is illuminated to the utmost in one half at least; after which he moves the telescopic tube until the vertical line dividing the field into two equal parts is rendered as narrow and well defined as possible, and the position of the flame is again looked to. The ivory lever is now moved forward, and the *inner notched* rim of the disc is rotated to the right or the left, until both halves of the field are equally obscure. The lever is next reversed, and the observer works the micrometer screw at the lower part of the disc, whilst he looks for a difference in the degree of illumination of the two halves of the field. If this does not occur, the latter was either too bright or too obscure. It may be rendered brighter by increasing and inverting the angle between the two rods (*c*, *d*) to which the polarisers are connected; and the less this angle, the less will be the variation with equal differences of adjustment. When the proper degree of illumination is obtained, a number of readings are taken with different adjustments.

The number of degrees to the nearest $\frac{1}{4}^\circ$, from the zero-point of the disc to that of the vernier, are read off; and then, proceeding in the same direction, the first line on the vernier which exactly corresponds with a division on the disc is noted. This is easily found by inspecting the divisions to the right and left of that one which is assumed to be correct. Both are on the *inner* side of the corresponding marks on the circle. The longer lines on the vernier correspond to 0.01°, the short ones to 0.005°. In practice, the adjustments should not differ by more than 0.005°.

The way in which the readings are taken may best be shown by an example:—Supposing the zero-point of the disc stood to the right of the zero-point of the vernier, and that, between the two, $\frac{3}{4}^\circ$ in addition to twenty long vernier marks and one short one had been counted, then $+ \frac{3}{4}^\circ$, 205 is written down. With further readings, the vernier marks only are noted, added together, and the average taken. Should the result again be 205, then

$$\tfrac{3}{4}^\circ = 0.75^\circ,\ 0.75^\circ + 0.205^\circ = + 0.955^\circ.$$

One long mark = 0.01°, 20 long marks = 0.200°.
One short mark = 0.005°, 1 short mark = 0.005°.

Finally, the zero-point is fixed by taking the tube out of the capsule *without otherwise altering the arrangement of the instrument.*

The field of vision is unequally illuminated, and the mark no longer distinct. The telescope is focussed to show the mark, the lever brought forward, the disc put in with the hand, the lever reversed, and the micrometer screw carefully adjusted. A number of readings is taken, and an average struck. Supposing this to be $= -2.045^\circ$ (*i.e.*, the zero mark for the present inclination of the polariser rods), the number is to be deducted from the original reading; thus, $+ 0.955 - (-2.045) = 0.955 + 2.045 = 3.0^\circ$.

If the urine was examined in a tube two decimetres long, then

$$2\,[\alpha]_D = 3.0^\circ, \text{ and } [\alpha]_D = 1.5^\circ$$

$[\alpha]_D$ being the specific rotating power for grape-sugar ($C_6H_{12}O_6$), is in this case $= +52.5^\circ$.

52.5° for 100 grm. in 100 cc.

1° for $\frac{100}{52.5}$ grm. in 100 cc.

1.5° for $\frac{100 \times 1.5}{52.5}$ grm. in 100 cc.

The percentage of sugar in the case taken would therefore be

$$\frac{100 \times 1.5}{52.5} = 2.85 \text{ per cent.}$$

Care should be taken throughout the observation that the position of the lamp is not altered, otherwise, varying results are obtained. During the whole process, therefore, the same position of the lamp and instrument must be maintained, and all readings should be taken continuously with one filling of the platinum cage.

By carefully observing these precautions, very accurate and reliable results may be obtained.

The instrument will serve also to show whether a liquid has the power of rotating light or not. In the latter case, the zero-point of the disc stands in the same lateral position to the zero-point of the vernier as the second pointer to the zero-point of the segment connected with the polariser (right or left), the deviation of the zero-point of the disc from that of the vernier being half as great as the deviation of the two rods.

2. Lævulosuria.—Fruit-sugar is found sometimes in conjunction with grape-sugar in the urine (*K. Zimmer, Seegen*).[195] The latter will then yield all the reactions of grape-sugar, including that with phenylhydrazin. It may happen under such circumstances that a specimen of the urine examined with the polarimeter will fail to rotate polarised

light to the right, or may even rotate it to the left; and from this fact the presence of lævulose may be inferred.

3. Lactosuria.—Milk-sugar occurs in the urine of women who are nursing.[196] Its recognition can be effected only by separation from the fluid (*Hofmeister*). An effort has been made with the phenyl-hydrazin method to transform this body in the urine into phenyl-lactosazon (*v. Jaksch* [197]), but as a test the expedient has not succeeded. *Rubner's* test for milk-sugar (p. 229), on the other hand, has some pretensions to practical value.

4. Inosituria.—Diabetic and albuminous urine is apt to hold a small proportion of inosite. This body must also be isolated for its detection. The best method for the purpose is that of *Cooper-Lane.*[198]

5. Dextrin.—Dextrin has been seen in the urine of diabetes (*E. Reichard* [199]), where it seemed to take the place of grape-sugar. In such cases Reichard found that the urine behaved with Trommer's test in all respects like a solution of dextrin, the originally blue fluid becoming first gradually green, then yellow, and sometimes dark brown.

6. Animal Gum.—*Landwehr* [200] has recently found in the urine a carbohydrate presenting a close analogy to members of the gum series. To this he has given the name of "animal gum," and he believes that it is a normal constituent of the fluid. The methods for its detection and isolation will be found in the original contribution on the subject. The statements there made are confirmed by *Wedenski.*[201]

Amongst other carbohydrates occasionally present in health and disease are maltose (*Le Nobel* [202]), and a left-rotatory body observed by *Leo* [203] in diabetic urine.

III. Choluria.—Of the bile constituents, the biliary acids and pigments chiefly concern us here. A third constituent of the bile, cholesterin, has never yet been found in the urine in jaundice, although it occurs in considerable quantities in other connections.

Hoppe-Seyler [204] has satisfactorily proved that biliary acids occur in the urine of jaundice; but their presence is of relatively little clinical interest, since they can be detected only by tedious chemical processes, but seldom suited to our purpose. None of the methods which have been suggested for the recognition of these bodies directly in the urine can be relied upon. That of *Mackay*, which is founded upon their physiological properties, is perhaps the most useful with which we are acquainted. Where the presence of bile acids in considerable proportion is suspected, resort may be had to the method for their detection in the blood (p. 50). The biliary acids, when isolated, or obtained as an alcoholic extract from the evaporation residue of the urine, may also be submitted to the furfurol test. To this end the fluid is treated with a few drops of a 1 per cent. watery solution of furfurol and sul-

phuric acid. The presence of biliary acids is shown by a red colouration.[205]

A fact of much greater practical interest is the occurrence of bile-pigments in the urine. This results from the comparative ease with which they may be recognised. Their manifestation implies, in the first place, that there has been obstruction of the bile-ducts in the liver, in consequence of which substances secreted by that organ have made their way into the lymphatics and the general circulation, from which they are subsequently eliminated by the kidneys. This is *hepatogenic jaundice*, the commonest form of choluria. The conditions which cause it are manifold and various. Of these, simple obstruction or narrowing of the bile-ducts is the most obvious. But when it is remembered that the pressure under which the bile is secreted is very slight, it will become apparent that other circumstances — as, for instance, one-sided immobility of the diaphragm—will tend to retard the propulsion of bile, and consequently to induce choluria. Such causes must be discriminated by physical examination, and on other grounds.

The bile-pigments of urine are not in all cases necessarily derived from the liver. It is conceivable that, with a quite normal biliary function, these bodies may be present, and they then owe their origin to blood-colouring matter which has undergone certain changes (p. 39); and these changes may take place either in the blood proper (*hæmatogenic jaundice*[206]) or supervene when the colouring matter has been discharged in the tissues (*Quincke's inogenic jaundice*).[207]

The author concluded from certain observations which he has recently made that the yellow discoloration which follows ecchymosis marks usually the formation of urobilin, and not the deposition of bile-pigments in the deeper layers of the skin.

It follows from what has been said, that the appearance of bile-pigment in the urine is a fact of very extended import, and caution must be observed in inferring from it the existence of hepatic disease. Still the latter is by far its more common association.

Urine containing bile-pigments is usually clear, deeply stained a yellow- or greenish-brown, and when shaken shows a yellow froth, even when these bodies are in very small proportion. The chemical tests for bile-pigments are very numerous, but it will suffice here to describe three, which alone, perhaps, are entirely reliable.

It has been claimed for the "cholecyanin test" of *Stokvis*[208] that it is the most sensitive of all; but of this the author has no experience. It must be borne in mind that bilirubin alone, of all the bile-pigments, occurs in fresh urine; the other pigments, biliverdin, bilifuscin, and biliprasin, being its oxidation-products.

1. *Gmelin's Test.*[209]—A small quantity of nitric acid containing nitrous acid is placed in a test-glass, and a little of the urine under

examination is poured in a separate layer upon its surface. To do this, care will be necessary, and it will be facilitated by slanting the test-glass so that the urine shall float on the surface of the acid. If bile-pigment be present, there is a play of colours at the point of contact between the acid and the urine, and a green ring in particular forms, indicating the production of biliverdin. Gmelin's test will not serve for urine which has been treated with alcohol, since that body, when similarly brought in contact with nitric acid, also yields a beautiful bluish-green ring (*H. Huppert*).[210]

In *Rosenbach's*[211] modification of Gmelin's test, the urine is passed through a filter, and on the latter a drop of nitric acid is allowed to fall, when the coloured rings will develop around it. This process affords a very sensitive test, but its results can be relied upon only when the filter-paper employed is known to be absolutely free from impurities (colouring matter) which might account for them.

Dragendorff's[212] method is to place a little of the urine on a plaster of Paris disc, and when the greater part has been absorbed, a drop of nitric acid is applied to the remainder. A parti-coloured ring forms around, in which the green tinge is prominent.

2. *Ultzmann's test*[213] is very serviceable in cases where the urine contains a considerable proportion of bile-pigment. The urine is mixed with solution of caustic potash (one part to three of water) in a test-glass, and hydrochloric acid added. The production of biliverdin by oxidation is indicated by the fluid assuming an emerald-green tint.

3. *Huppert's method*[214] serves for the detection of the merest traces of bile-pigments. 8 to 10 cc. of urine are treated with milk of lime, and the resulting precipitate is removed by filtration, and treated with sulphuric acid and alcohol in a test-glass. Sulphuric acid is added until the solution has an acid reaction. It is then boiled, when the precipitate is decolorised, if bile-pigment is present, and the liquid assumes a green tint. With similar treatment urine abounding in indican deposits a bluish-grey precipitate at the outset, but with the subsequent process not a green, but, if any, a yellow or reddish colour develops. Urine which contains urobilin, on the other hand, is made in this way to exhibit a deep-rose colour (p. 241).

Ehrlich[215] has devised the following method for the detection of bilirubin in the urine. The latter is mixed with its own bulk of dilute acetic acid, and, drop by drop, is added a mixture consisting of 1 grm. sulphanilic acid, 15 cc. hydrochloric acid, and 0.1 grm. nitrite of sodium to the litre. A dark discoloration takes place, which on the further addition of glacial acetic or other acid passes to a characteristic violet colour (bilirubin).

IV. Urobilinuria.—*Jaffé*[216] was the first to discover urobilin in the urine. It seldom exists pre-formed[217] in the recently passed healthy fluid, but the latter holds a chromogen (see p. 173), which yields urobilin on the addition of acid. This body is present in large amount in the urine of certain morbid states, amongst which may be mentioned fever, and the various affections which are characterised by the disintegration of red blood-corpuscles, as scurvy (*Kretschy*[218]), and in Addison's disease (*Kummer*).[219] It often happens, in cases which would be described as slight jaundice, that a very dark-coloured urine is passed, and this is found on examination to be free from bile-pigments, but abounding in urobilin (*Gubler, Gerhardt*[220]). This so-called "urobilin" jaundice occurs but rarely in connection with hepatic disease, most frequently with hepatic cirrhosis.

It is further a fact of great clinical interest that the excretion of considerable quantities of urobilin has been observed to attend on intracranial hæmorrhages [*Berymann, Kunkel*[221]], hæmorrhagic infarction, retro-uterine hæmatocele, and extra-uterine pregnancy (*Dick*).[222] Apart from its occasional association with liver complaints, the author's experience has been that urobilinuria occurs most commonly in the course of extensive cutaneous hæmorrhages due to scurvy, carcinoma, the hæmorrhagic diathesis, &c.; extravasation under such conditions was constantly followed by the appearance of urobilin in the urine, and this became more marked as the process fell into abeyance, thus suggesting the inference that the blood colouring-matter discharged into the cuticular tissues was again absorbed and eliminated by the kidneys in the form of urobilin. The individuals in whom this process was going on commonly exhibited a pronounced yellowness of the skin. In such of these cases as afforded an opportunity for post-mortem examination, the bile-ducts were invariably found unimpeded, and during life the urine was free from biliary pigments. In the cases of urobilinuria which have come under the author's notice, the skin was very commonly tinged yellow (jaundiced?), but this was not without exception.

Recent investigations by *Leube*[223] have made it probable that under some circumstances bilirubin is oxidised to urobilin in the kidney. If this is so, we obtain a new form of urobilinuria.

Urine which contains much urobilin is characterised by its very dark colour; but this does not suffice to distinguish it, inasmuch as other substances, and notably an abundance of the antecedent of indigo, will impart the same quality to the water. Moreover, it will sometimes furnish a beautiful yellow froth like that of jaundice. Such urine has the property of displaying fluorescence in presence of ammonia and zinc chloride. For the detection of urobilin *Gerhardt*[224] suggests that a chloroform extract of the urine should be treated with solution of

iodine, and caustic potash added, when a beautiful green fluorescence develops. The author has adopted in about eighteen cases of urobilinuria the following method:—The urine is submitted to *Huppert's* test for bile-pigments (p. 239). If urobilin be present in considerable proportion, the precipitate is coloured a brownish-red, and becomes decolourised on boiling with sulphuric acid, while the fluid assumes a brownish or pomegranate-red tint.

The urine in this condition may be freed from colouring matter by æther or amylic alcohol. Its spectroscopic characters are remarkable. When acid, if rich in urobilin, it displays a distinct absorption-band in the green and blue between Fraunhofer's lines *b* and *F* (fig. 111), and usually extending with diminished intensity beyond *F*. When alkaline, a less well-marked band is seen, midway between *b* and *F* (fig. 112).

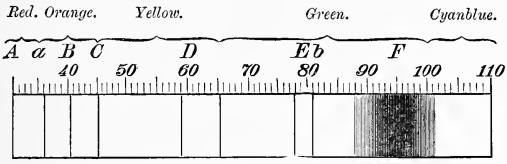


Fig. 111.—Spectrum of Urobilin in Acid Urine.

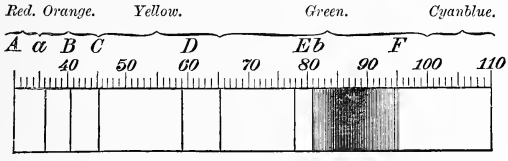


Fig. 112.—Spectrum of Urobilin in Alkaline Urine.

The quantitative estimation of urobilin may be effected by means of *Vierordt's*[225] spectrophotometer. For its separation, *Jaffé's*[226] method, or that of *Méhu*[227] (see p. 163), may be employed.

V. Æther-Sulphuric Acids and their Derivatives (*Indigo-Blue, Phenol, Skatol, Parakresol, Pyrocatechin, Hydrochinon*) and the Aromatic Oxy-Acids.

(*a.*) **Indicanuria.**—Indigo (indigo-blue, indigotin), as such, is rarely present in the urine, usually in decomposed urine, and hardly ever so plentifully as to impart its colour to the fluid. It may always, however, be obtained from urine as a product of the decomposition of salts of indoxyl-sulphuric acid (potassium indoxyl-sulphate).[228]

Indol, a regular product of bacterial putrefaction of albumin (see p.

162), is the basis of indican (indoxyl-sulphuric acid).[229] It is oxidised to indoxyl within the system, and by combination with the sulphuric acid present forms indoxyl-sulphuric acid. The decomposition of indoxyl-sulphuric acid yields, besides indigo-blue, other substances of similar character, such as indirubin; but to these no practical interest attaches in the present state of our knowledge.[230]

With reference to the clinical import of indicanuria, it must be borne in mind that the quantity of indoxyl-sulphuric acid formed varies in health with the food ingested, and it is increased especially by animal diet.

Apart from this, an undue proportion of indican in the urine is a fact of pathological interest, and there are certain diseases in which indoxyl-sulphuric acid is regularly produced in excess.

It was formerly believed that starvation and wasting diseases were attended with the separation of indican (*Senator*[231]); but more recent observation[232] has shown that this is dependent on the fact that in such diseases albuminous putrefaction takes place in the alimentary canal, and in consequence there is an increased production of indol, the antecedent of indican. The presence of indican in the urine is very often a sign of intestinal putrefaction, and its quantity in certain cases varies with the activity of that process.[233] It may also accompany the decomposition of albumin in other cavities. Thus, in a case of pleurisy with abundant unhealthy exudation, the author has found a profusion of indican in the urine, and when this manifestation arises in the course of peritonitis, it may be taken as an evidence of the character of the disease, and of the formation of putrid pus.

In general, therefore, the appearance in the urine of large quantities of indican implies that abundant albuminous putrefaction is progressing actively in some part of the system. Caution must be observed in further narrowing the inference as to diagnose a gangrenous suppuration, for instance, since in simple constipation a notable indicanuria will very often arise.

It may be remarked here that the deep brown colour which usually belongs to urine rich in indican is not due directly to the presence of indoxyl-sulphuric acid, but depends upon the higher oxidation products of indol which accompany it. These bodies bear to indoxyl-sulphuric acid the same relation that the brown, green or black colouring matters of carbolic urine do to phenol-sulphuric acid.

Detection of Indican.—The methods employed for this purpose proceed upon the principle of splitting up the indoxyl-sulphates of the urine, and obtaining from them a coloured product—indigo-blue.

Jaffé's Test.[234]—A few cc. of the urine are treated with an equal quantity of hydrochloric acid, and, drop by drop, a solution of some

hypochlorite is added by means of a glass pipette, and shaken up with the fluid. The chromogen formed by decomposition of indoxyl-sulphuric acid is oxidised into indigo-blue. Care must be taken that hypochlorites are not in excess, since this would alter and bleach the indigo-blue. *Stokvis* [235] recommends the admixture of a little chloroform in the process, with the object of dissolving the indigo-blue as it forms. The chloroform solution then takes a blue colour [and the colouring matter is obtained as a deposit after evaporation].

Weber's Test.[236]—Thirty cc. of urine are mixed with an equal quantity of hydrochloric acid, 1–3 drops of dilute nitric acid added, and the mixture boiled. The fluid assumes a dark colour. If allowed to cool and then shaken up with æther, the presence of indigo-blue is shown by the formation of a blue froth on the surface, while the æther exhibits a rose or violet tint.

Quantitative Estimation.—The methods of *Jaffé* and *Salkowski* are the most useful. Their principle is the same as that of the tests for the presence of indican.

Salkowski's [237] *Colorimetric Process* is perhaps the best. A rough analysis is first effected by determining the quantity of chlorinated lime solution with which indigo forms in greatest abundance. If in this way it is found that the urine contains much indican, 2.5–5 cc. are diluted with water to 10 cc., while if there be but little indican present, 10 cc. of undiluted urine are taken as the basis of the experiment. In either case an equal quantity of hydrochloric acid is added, and that proportion of chlorinated lime solution which was found in the preliminary reaction to be required. The mixture is then neutralised with caustic soda, and carbonate of soda added to make it alkaline. The indigo-blue which forms is collected in a filter, and then washed with water until it no longer exhibits an alkaline reaction, when it is dried and repeatedly extracted by heating with chloroform until the latter ceases to colour with it. The chloroform extract is then, by the addition of chloroform, made up to a quantity expressed by a round number of cc., placed in a glass vessel with parallel sides, and the intensity of its colour compared with that of a freshly prepared chloroform solution of indigo-blue of known strength. To one or other of these, as required, more chloroform is added, until their tint is adjudged equal. From the known constitution of the standard the quantity of indigo-blue derived from the urine (2.5–5 or 10 cc.) taken may be also known, and its percentage may be readily calculated.

From the urine passed in twenty-four hours under ordinary conditions of diet 5–20 mgrm. of indigo-blue can be obtained on an average.

There are certain other aromatic derivatives of the urine which will engage our attention here, both because they are chemically allied to

indoxyl-sulphuric acid, and also because pathologically their production is apt to coincide with the manifestation of that substance.

(*b.*) **Skatoxyl-Sulphuric Acid.**—This body results from the skatol of the fæces (*Brieger*[238]). It is assumed that skatol, by a process analogous to that undergone by indol, is oxidised to skatoxyl within the body, appearing in the urine as skatoxyl-sulphuric acid. It is probable that the red colour which develops in the urine in the presence of acids is to some extent due to decomposition products of this substance.[239]

(*c.*) **Parakresol- and Phenol-æther-Sulphuric Acid, Paroxyphenyl-Acetic Acid, and Paroxyphenyl-Propionic Acid.**—The other members of the aromatic group which occur in human urine in combination with sulphuric acid are phenol (carbolic acid), parakresol, pyrocatechin, and hydrochinon. To the latter we shall have need to refer again. The aromatic oxy-acids, paroxyphenyl-acetic acid, and paroxyphenyl-propionic (hydroparacumaric[240]) acid are also found there. The methods by which these bodies may be detected are highly interesting and clinically instructive.

Salkowski[241] has shown that the urine of patients suffering from ileus and peritonitis, in addition to a large percentage of indican, contains also a considerable proportion of phenol-forming substance, and *Brieger's*[242] experiments have proved that the elimination of the antecedent of indigo (indoxyl-sulphuric acid) on the one hand, and that of the phenol-producing substances (phenol-, parakresol-æther-sulphuric acid) with the aromatic oxy-acids on the other, bear to one another no constant relation as to activity; and that author found that in diphtheria, scarlatina, and facial erysipelas phenol was formed in greatly increased quantity, whilst in typhoid, relapsing and intermittent fevers, small-pox, and meningitis it could be obtained but very sparingly from the urine. These statements are borne out by others.[243]

Again, in all cases where albuminous putrefaction is actively progressing in the intestine or other organs, in addition to the salts of indoxyl-sulphuric acid phenol is increased in the urine, and in general together with phenol the other members of the aromatic group become evident in connection with pulmonary gangrene, putrid bronchitis, fœtid pleuritic exudation, and decomposition generally throughout the body.

Detection of Æther-Sulphuric Acids.—For this purpose the urine is first treated with barium chloride in excess to precipitate simple sulphuric acid and then boiled with hydrochloric acid. If æther-sulphuric acid be present, it is decomposed with the formation of the uncombined acid. This combines with the barium present to form sulphate of barium, and a white precipitate is deposited.

Detection of the Aromatic Oxy-Acids.—Twenty cc. of urine are treated

with hydrochloric acid, and heated for some time on the water-bath to expel the volatile phenols. It is then allowed to cool, and repeatedly extracted with æther. The æthereal extract is shaken up with a weak solution of carbonate of soda. The oxy-acids are taken up by the latter, whilst the phenols present are retained by the æther. The alkaline solution is now acidulated with sulphuric acid and extracted with æther. The latter is allowed to evaporate, and the residue dissolved in water is submitted to Millon's test (p. 212). A red colouration shows the presence of aromatic oxy-acids.

In a similar manner an approximate quantitative estimation of these bodies may be effected.[244]

The quantitative estimation of the phenols (phenol, parakresol) is conducted in the manner to be described at p. 246, and their presence may be determined by the tests given at p. 122 and p. 162. For the comparative investigation of these substances the original work of *Brieger*[245] may be consulted.

Quantitative Estimation of Æther-Sulphuric Acid.—The percentage of this body in the urine may be best determined by *Baumann's*[246] method as modified by *Salkowski*.[247]

To 200 cc. of urine is added a like quantity of alkaline barium chloride solution (two parts saturated solution of baryta, and one part of solution of chloride of barium saturated in the cold). The mixture is allowed to stand for some minutes and then passed through a thick filter which has been carefully dried. Of the filtrate, *which must be perfectly clear*, 100 cc. are taken and rendered strongly acid with 10 cc. of hydrochloric acid (sp. gr. 1.12), then boiled and heated on the water-bath until all the precipitate which forms has settled. The beaker may be heated on an iron plate coated with asbestos, and allowed to remain till its contents are cold. The precipitate is next collected and placed on a filter of Swedish paper which has previously been washed with dilute hydrochloric acid, and care must be taken that the filter is not allowed to empty itself entirely during the process. The instrument described at p. 255 is very suitable for the purpose. With the aid of a glass rod protected with a ring of india-rubber, and rinsing with boiling water, the entire precipitate is placed on the filter, and is there washed with boiling water until the filtrate which passes through fails to give a precipitate with dilute sulphuric acid, thus showing the absence of free chloride of barium. Should it happen that the fluid passing through the filter is turbid, this may be due to the presence of soluble substances, as phenols produced by the decomposition of the compound acids. To ascertain their character in this case, the cloudy filtrate is placed in a beaker on the water-bath heated to boiling-point, when, if the turbidity be due to phenols, these pass off in vapour and leave

the fluid clear. Where, on the other hand, the appearance is caused by the barium precipitate having passed through the filter, the turbidity will not be removed in this way, and the experiment is spoiled. The precipitate is next washed with boiling alcohol, and finally with æther, and together with the filter-paper placed upon a platinum crucible of known weight and heated for a long time. After this the platinum crucible is raised to a white heat, allowed to cool, and then weighed again. The calculation is made as follows:—233 parts by weight of sulphate of barium correspond to 98 parts by weight of sulphuric acid (H_2SO_4), and consequently the quantity of sulphuric acid in 100 cc. of the urine may be computed by the formula—

$$x = \frac{98}{233} \times M = 0.4206 \times M$$

Where

x = the quantity of sulphuric acid required.

M = the quantity of barium sulphate found.

If it be desired to determine the total quantity of the sulphuric acids (simple and compound), so as to find out their respective proportions in the urine, the latter is filtered. Another 100 cc. are taken, and treated with 10 cc. of hydrochloric acid (sp. gr. 1.12), then boiled for fifteen minutes and chloride of barium solution added in excess. The remainder of the process is that described above. The difference between the total quantity and that of æther-sulphuric acids obtained expresses the proportion of simple sulphuric acid in the urine.

For the methods of estimating sulphur when present in the urine in other forms see pp. 268, 271.

Quantitative Estimation of Phenols.—The phenols (phenol and para-kresol) which have passed over in the distillation of a known quantity of urine previously acidulated are estimated in the form of tribromo-phenol by Landolt's method, with the precautions suggested by *Baumann* and *Brieger*.[248]

One-fourth of the urine passed in twenty-four hours is mixed with one-fifth its bulk of hydrochloric acid and distilled. Distillation is continued as long as the distillate continues to colour with bromine water, and filtered. All the fluid which has passed through—including that tested during the process—is now treated with bromine water until a permanent yellow colour is attained. The precipitate is allowed to settle for two or three days, when it is separated on a filter which has been weighed and carefully dried over sulphuric acid; it is then washed with bromine water and dried over sulphuric acid in the dark until it has acquired an approximately constant density. It is then weighed on the filter, and the difference between the result and the weight of the filter recorded expresses that of the tribromo-phenol

formed. From this the quantity of carbol in the urine may be estimated thus:—331 parts by weight of tribromo-phenol, correspond to 94 parts by weight of carbol, and the following formula results:—

$$x = \frac{94}{331} \times M = 0.2839 \times M$$

Where

x = the quantity of carbol required.

M = the quantity of tribromo-phenol found.

This method may also be employed for the analysis of vomited matters in cases of carbolic acid poisoning (compare p. 122).

J. Munk has computed that the quantity of phenols excreted with the urine averages 0.017 to 0.051 grm. in the twenty-four hours. For purposes of more accurate analysis it is important to determine the quantity of indoxyl-sulphuric acid and æther-sulphuric acids eliminated in the manner already indicated (p. 243), and to remove the analogous substances generated in intestinal decomposition by means of a calomel purge.[249]

(*d.*) **Pyrocatechin.**—This body, like the others, occurs in the urine only in combination with sulphuric acid; and it is, if not an invariable, at all events a very frequent constituent of that fluid.[250] *Boedeker*,[251] who first discovered it there, gave it the name of alcapton. It was afterwards found in abnormal quantity in the urine of a child,[252] and again in certain cases of phthisis.[253] The urine containing it is characterised by the fact that it is colourless when passed, but becomes dark after exposure to the air; and the change is expedited by the addition of caustic potash. When boiled with hydrochloric acid, it becomes a powerful reducing agent. An ammoniacal solution of silver exposed to it will quickly deposit metallic silver in the cold.

These properties, however, are not sufficient to determine the presence of pyrocatechin. To do this it must be isolated, and the following method is the best for the purpose.[254] The urine is evaporated to one-fourth its volume on the water-bath, filtered, and the filtrate boiled with excess of sulphuric acid. It is then allowed to cool and repeatedly shaken up with æther, the æthereal extracts collected, the æther distilled off, the residue neutralised with carbonate of baryta, and again extracted with æther. Pyrocatechin passes over in the æthereal extract, and when the æther is distilled off, it remains as a more or less pure crystalline substance. If the character of the latter is not sufficiently apparent, it may be re-crystallised from benzol in the form of prisms belonging to the tetragonal system. If some of these be dissolved in water in a watch-glass and a few drops of a very dilute solution of perchloride of iron be added, an emerald-green colour develops, and this changes to violet on the addition of a little ammonia.[255]

(*e.*) **Hydrochinon.**—It has been ascertained by *Baumann* and *Preusse*[256] that hydrochinon appears in the urine after carbolic acid poisoning, and these authors believe that to its presence the dark colour of that fluid after the exhibition of carbolic acid is due. It is always in the form of æther-sulphuric acid in the urine, and the process for its detection is the same as that for pyrocatechin.[257]

The crystals of hydrochinon belong to the rhombic system, and they crystallise readily from their solution in toluol.

According to *Baumann* and *Preusse*,[258] when rapidly heated in an open test-tube, hydrochinon forms violet fumes, which condense as an indigo-blue sublimate, and in the application of this property we possess a very sufficient test for its presence.

VI. Melanuria.—The urine of persons suffering from pigmented tumours sometimes contains a substance to which the name of melanin has been given, but of which the chemical constitution is not yet sufficiently established. It is sometimes held in solution, and more rarely suspended in the form of small granules. It very seldom happens that the fluid has a dark colour when passed, but it generally blackens intensely when submitted to oxidising agents (sulphuric and hydrochloric acids and ferric chloride). This fact would point to the conclusion that the body in question is not melanin, but a chromogen—melanogen—analogous to that which precedes the formation of urobilin. Such urine turns dark on exposure to air. The pigment can be partially separated from the urine by means of acetate of lead or perchloride of iron. It is insoluble in cold alcohol, æther, and acetic and dilute mineral acids. It is soluble in boiling concentrated mineral acids, in boiling lactic and acetic acids, in concentrated solutions of caustic potash and soda, and in ammonia. It contains iron, sulphur, and nitrogen. The most sensitive test for the presence of melanin is the addition of bromine water (*Zeller*[259]), which causes the urine to deposit a yellow precipitate, which gradually blackens.

More recent experience has shown that a fairly concentrated solution of perchloride of iron serves well to detect its presence (*v. Jaksch*).[260] A few drops of this reagent will cause the fluid to turn grey; and if more be added, a precipitate of phosphates falls, carrying the colouring matter with it, and again dissolves with an excess of the solution.

Sodium nitro-prusside with caustic potash and acetic acid gives a deep blue colour (*Thormählen*[261]), which depends probably on the formation of soluble and insoluble Berlin-blues (*v. Jaksch*).

In connection with this subject the reader may refer to the opinion of *Krukenberg*[262] and *Salkowski*[263] (see p. 261) that in *Weyl's* test for kreatinin boiling with acetic acid similarly produces Berlin-blue.

The latter, however, cannot always be obtained by the action of the nitro-prusside salt on melanin isolated from the urine, and the reaction must *not* be regarded as a test for melanuria, or only when other tests (and especially that with perchloride of iron) have shown the presence of melanin or melanogen. Moreover, the Berlin-blue reaction can be obtained in urine which is *free from melanin*. Thus, in the case of children suffering from prolonged constipation, it has been had at the same time that the fluid was rich in acetone or diacetic acid and indoxyl-sulphuric acid (*v. Jaksch*), and in a case of diabetes by *Dreschfeld*,[264] when its nature and the presence of the substances just named were probably established. It would appear, therefore, that in these conditions also a body is present which gives Berlin-blue with nitro-prusside compounds. Possibly this is indol. Investigations with that body derived from a preparation of picrate of indol gave the same result (*v. Jaksch*). The practical significance of this condition is greatly limited by the fact that the urine may contain a large quantity of melanin in wasting diseases, whilst that derived from individuals suffering from melanotic cancer or sarcoma may be entirely free from it. Nevertheless, as an adjunct in diagnosis, the tests given are of undoubted utility.[265]

VII. Acetonuria.—Normal urine contains traces of acetone (*physiological acetonuria*, *v. Jaksch*[266]), but this body occurs in excessive proportion under certain morbid conditions (*pathological acetonuria*). In association with diseases we may distinguish (1) febrile acetonuria; (2) diabetic acetonuria; (3) acetonuria accompanying certain forms of cancer independently of inanition; (4) acetonuria of starvation; (5) the production of acetones in psychoses; (6) acetonuria as an expression of auto-intoxication. The commonest of these forms is febrile acetonuria. It does not belong especially to any particular fever.[267] In connection with diabetes the appearance of acetone in the urine indicates an advanced stage of the disease, but does not otherwise affect the prognosis. Of greater consequence[268] are those cases in which much acetone is found in connection with grave symptoms of cerebral irritation, less often of depression. Acetonuria existing alone (auto-intoxication with acetone) tends to a favourable termination (*v. Jaksch*).[269] Finally, it should be noticed that recent researches have shown that an abundance of nitrogenous food tends to the production of acetonuria.

Detection of Acetone.—A rough test for acetone is that of *Legal*. A quantity of the urine (several cc.) is treated with a few drops of a freshly-made and somewhat concentrated solution of sodium nitro-prusside, and with a moderately strong solution of caustic soda or potash. The fluid develops a red colour, which rapidly disappears, and

if acetone be present, gives place to purple or violet-red on the addition of a little acetic acid. In the absence of acetone the purple-red tint does not form on the addition of acetic acid.

For purposes of greater accuracy it is necessary to distil the urine, and to apply to the distillate the tests presently to be described. To do this, one-half to one litre of the urine may be taken, and a little phosphoric acid may be placed with it in the retort to prevent the evolution of gases. Of the distillate 20–30 cc. may be taken and tested with—

(1.) *Lieben's Test.*—To several cc. a few drops of iodo-potassic iodide solution and caustic potash are added. If more than a trace of acetone be present, an abundant precipitate of iodoform crystals is deposited. This test is very reliable, and will serve even for the detection of traces of acetone.

(2.) *Reynolds' Test.*—This depends on the property which acetone possesses of promoting the solution of recently-formed mercuric oxide. It is conducted as follows:—The yellow precipitate (mercuric oxide) obtained by the reaction of mercuric chloride with an alcoholic solution of caustic potash is added to the distillate from the urine, which is then filtered, and to the clear filtrate sulphide of ammonium is cautiously added. If acetone be present, some of the mercuric oxide will have dissolved and a black ring (sulphide of mercury) forms at the plane of contact with the ammonium sulphide.

Legal's Test, already described, may be applied also to the urinary distillate, but it is less reliable than the others, since parakresol, which also passes over in distillation, exhibits a similar reaction.[270]

VIII. Diaceturia.—By the term diaceturia is meant the condition in which diacetic acid appears in the urine. It is always pathological,[271] and occurs in diabetes (*Gerhardt*) and fevers (*v. Jaksch, Deichmüller, Seifert*), and also idiopathically as a form of auto-intoxication. It is most common in children as a concomitant of fever, and is then generally devoid of serious significance, but in adults it is a symptom of grave import. In febrile and diabetic states the development of diaceturia commonly forebodes the advent of coma.

Urine holding diacetic acid is always rich in acetone, and in presence of perchloride of iron develops a Bordeaux-red. This property, however, does not serve to distinguish the presence of the former body, since it belongs equally to a number of substances which are apt to exist in the urine.[272] For its detection the following process may be adopted.

To the urine a fairly-concentrated solution of perchloride of iron is cautiously added, and if a phosphatic precipitate forms, this is removed by filtration and more of the perchloride of iron solution supplied. If the Bordeaux-red colouration appears, one portion of the urine is

boiled, whilst another is treated with sulphuric acid and extracted with æther. If now the urine which has been boiled shows little or no change, whilst the perchloride of iron reaction in the æthereal extract is no longer evident after 24–48 hours; and if at the same time (on testing the urine directly and its distillate) it is found to be rich in acetone, the condition may be inferred to be that of diaceturia.

IX. Lipaciduria.—By this term is meant the condition in which volatile fatty acids are found in the urine (*v. Jaksch*,[273] *v. Rokitansky*[274]). These bodies occur there in traces normally, especially formic, acetic, and butyric acids; and they may be derived from healthy urine in considerable quantity by the use of oxidising agents. They are also a product of alkaline fermentation.[275]

As a manifestation of disease, on the other hand, they are often present in quantity in the simple urine. Thus, in the urine of fevers, of hepatic diseases affecting the proper structure of the liver, and in diabetes, formic, acetic, butyric, and recently also propionic acid have been detected.

There is no special diagnostic significance attaching to this condition; in general, it is determined by the same causes which produce febrile acetonuria.

For the detection of fatty acids the urine is distilled with phosphoric acid, and the distillate carefully neutralised with carbonate of soda, evaporated to dryness on the water-bath, the residue extracted with boiling alcohol, filtered, again evaporated, dissolved in water, and the solution submitted to the tests mentioned at p. 161. The principal reactions are shortly recapitulated here.

1. A little of the urine is treated with sulphuric acid and alcohol. An odour of acetic æther indicates the presence of acetic acid.

2. To another portion perchloride of iron is added. The specimen assumes a red tint, which disappears on boiling, and a rusty precipitate remains.

3. The addition of nitrate of silver causes a white precipitate, which rapidly blackens if formic acid be present.

With reference to the appearance in the urine of other organic acids, see pp. 279–280.

X. Lipuria.—Small quantities of fat are often seen in the urine of chronic nephritis with a very fatty state of the kidney (see pp. 185, 202), in phosphorus-poisoning,[276] and diabetes mellitus. Fat in large proportion was found by *Ebstein*[277] in a remarkable case of pyonephrosis.

Fat is also a common manifestation in chyluria, and it is a physiological constituent of the urine of pregnant women.

Its presence is sufficiently apparent. The urine containing it is usually very turbid, and clears when shaken up with æther. It is apt also to hold globules of fat, which are easily recognisable by their

powerful refracting properties; and it is not uncommon for this substance to occur in the form of needles, as it does in the fæces (p. 156), especially in connection with chronic nephritis and septicæmia.[278]

XI. Chyluria.—By this term is meant the simultaneous appearance at intervals of fat and albumin in the urine, apart from the manifestation of other morbid constituents, such as casts, renal epithelium, &c. The sediment, however, usually contains red and white blood-corpuscles in small numbers.

The urine under these circumstances tends to form coagula of fibrin on standing, and occasionally it gelatinises throughout. Hitherto chyluria has been met with almost exclusively in the tropics, and in persons who have lived there for a long time, and it has been shown by *Wucherer* and *Lewis* (p. 193) to depend upon the invasion of the urinary tract by Filaria sanguinis hominis. The embryo of this parasite is generally to be found in the urine; and the chemical investigations of *Grim*[279] make it appear that in the majority of cases the abnormal condition of the urine is due to unnatural communications between the lymphatics and urinary passages affected by filaria. The subject, however, needs further elucidation, inasmuch as chyluria is occasionally observed in persons who have never lived in the tropics.[280]

Langgaard[281] has detected large quantities of cholesterin in the urine in a case of chyluria.

XII. Oxaluria.—It has been stated already that oxalic acid occurs in healthy urine, but it is subject to very great increase in certain morbid states, and the condition is then called oxaluria.

Oxalates may remain in solution in the urine, and it is important to be able to determine absolutely the quantity of oxalic acid present as such. This can be done by a modification of *Neubauer's* method (*Förbringer* and *Czapek*).[282]

Quantitative Estimation of Oxalic Acid (*Neubauer's Method*).[283]—The urine passed within twenty-four hours is accurately measured, and treated first with calcium chloride and ammonia, then with acetic acid until it has a slightly-acid reaction, and afterwards a little alcoholic solution of thymol is added to restrain the development of micro-organisms. The mixture is allowed to stand for some time, when the white precipitate which forms is separated on a filter, and (together with the latter) is placed in hydrochloric acid, gently heated, the fluid filtered off, and the filter washed with water until it has no longer an acid reaction. The collected filtrate is then evaporated to a small bulk in a capsule on the water-bath, then placed in a strong glass cylinder, and the capsule in which it was evaporated is washed with dilute hydrochloric acid and water, the washings being added to the fluid in the

cylinder. Ammonia solution is then poured upon the surface of the latter, and the whole is tinted with a few drops of tincture of litmus. The mixture is allowed to stand for a considerable time. The precipitate which has formed is then obtained on a so-called ash-free filter, the ash constituent of which has previously been accurately ascertained, and the oxalate (of lime) which adheres to the walls of the cylinder is removed on a glass rod guarded with an india-rubber ring, and added to the precipitate on the filter. The latter is next freed from chlorine by washing with water, and then rinsed with acetic acid. The filter is then dried, and ignited on a platinum crucible, which is heated to a constant weight in the blow-pipe flame. By this means oxalate of lime is changed into lime. Now as 56 parts of lime correspond to 90 parts of oxalic acid, the quantity of the former obtained when multiplied by 1.6071 shows the quantity of oxalic acid in the urine taken.[284]

In healthy urine the amount of oxalic acid passed in twenty-four hours is 0.02 grm. (*Fürbringer*).

An excess of oxalic acid is occasionally found in diabetes, and especially when the proportion of sugar diminishes (vicarious oxaluria[285]).

Oxaluria is also known as an affection *sui generis* (oxalic acid diathesis, idiopathic oxaluria), (*Cantani*).[286]

It must be admitted that our knowledge of this condition as a clinical symptom is very defective, but the author's experience induces him to adopt the conclusion of *J. Beybie*[287] and *Cantani*, that there are certain complaints, characterised by pains in the back and loins and attended with rapid emaciation, in which the only objective symptom besides is an excessive elimination of oxalic acid with the urine.

XIII. Cystinuria.—This is a condition of rare occurrence, and clinically of little importance, since it is only accidentally by the formation of calculi that it gives rise to trouble. It is usually chronic in its course. It should be mentioned that *Ebstein*[288] has found cystin concurrently with albumin in the urine of acute articular rheumatism (comp. p. 198).

XIV. The Uric Acid Diathesis.—Although the deposition of a very abundant sediment of urates in the urine does not warrant the inference that uric acid is excreted in excess, there is no doubt that there are certain processes in the system, the chief evidence of which is such an increase in the elimination of uric acid, and it is important to possess the means of estimating this condition.

Many methods have been devised for this purpose, as those of Fokker and Salkowski, and in recent years by *Haycraft*[289] and *Czapek*.[290] That of *Fokker*,[291] as modified by *Salkowski*,[292] depends upon the comparative insolubility of urate of ammonia. Those of *E. Salkowski*[293] and of *E.*

Ludwig[294] are based on the estimation of the almost insoluble double silver salt of uric acid.

Ludwig's process has the advantage that it can be carried out completely in twelve to fourteen hours, and it is further serviceable as a qualitative test for uric acid in the other secretions, and in the blood as well as in the urine.

For its application the following solutions are needed:—

i. *An Ammoniacal Silver Solution.*—This is prepared by dissolving 26 grms. nitrate of silver in distilled water, and adding ammonia until the brown precipitate thrown down at first is again dissolved. The fluid is made up to a litre, placed in a well-stoppered flask, and protected from the light.

ii. *A Magnesia Mixture.*—A hundred grms. of crystallised magnesium chloride are dissolved in water, and a large excess of ammonia added, and then ammonium chloride until the precipitate (magnesium hydrate) is entirely dissolved. The fluid so derived should be tolerably clear. It is made up to a litre and placed in a stoppered bottle till required.

iii. *Solution of Sodium or Potassium Sulphide.*—Fifteen grms. of caustic potash or 10 grms. of caustic soda are dissolved in a litre of water, and one-half of the fluid is saturated with sulphuretted hydrogen, after which the other half is added to it. The potash or soda used must be entirely free from nitrates and nitrites, and to this end it is well to use caustic soda prepared from metallic sodium.

A hundred or 200 cc. of urine are measured off in a dry glass cylinder and carefully poured into a beaker of 200–300 cc. capacity. Ten or 20 cc. (according as 100 or 200 cc. of urine has been taken) each of solutions i. and ii. are mixed together in a measure-glass, and ammonia is slowly added until the precipitate is dissolved. The clear fluid is then poured from the cylinder in which the urine was measured and added to the latter in the beaker-glass, and the mixture stirred for some time. The precipitate which forms is allowed to stand for half an hour or an hour, after which it is placed with the fluid on a filter, and two or three times the quantity of water, to which a little ammonia has been added, is supplied. For this purpose Ludwig employs an aspirator, but it is not necessary, since filtration proceeds rapidly enough without it.

The precipitate and the filter together are placed in the beaker, and 10 or 20 cc. (according to the quantity of urine taken) of solution iii., diluted with an equal quantity of water, is heated to boiling in a flask, added to the precipitate in the beaker, and the mixture frequently stirred, 40 cc. of boiling water supplied, and the mixture heated over a flame until it begins to boil. It is repeatedly stirred while allowed to cool, and passed through a filter, which is afterwards washed two or

three times with boiling water and collected in a large capsule. The filtrate is treated with hydrochloric acid until it has a feebly acid reaction, and is then concentrated on the water-bath to a volume of 10–15 cc. Uric acid begins to separate at this point in crystals, which are often of a beautiful white colour.

The best plan is to continue evaporating, without regard to the quantity of fluid remaining, until the point is reached when uric acid begins to separate from the hot solution.

The fluid is now allowed to cool for an hour, when the separation of uric acid will be completed. The precipitate is brought upon a Ludwig's filter arranged with glass-wool. This instrument consists of a glass tube about 14 cm. long and 2 cm. in diameter in its upper point, growing rapidly narrower below, and constricted to little more than capillary calibre at a point 4 cm. from its lower end (fig. 113). The lower end is cut off obliquely. The tube is packed from the point of constriction upwards as far as the beginning of the broad part with glass-wool, which is best introduced by means of a slender glass rod, and in such a manner that the obstruction is densest below, and less compact as it proceeds upwards.

To facilitate this, the glass-wool with which the funnel is to be blocked may be previously moistened with a little æther.

The upper end of the instrument is closed with a ground-glass stopper.

When arranged with glass-wool as described, the whole is dried at 110° C., allowed to cool, and weighed.

The filter is fixed in a suitable support, and the fluid with the uric acid precipitate is placed upon it. The filtrate is used to wash out the uric acid from the capsule in which it was formed, and this is repeated until no trace of the uric acid is left in the latter, the whole having been placed on the filter. Finally, the latter is washed repeatedly with a little water, and best by means of an aspirator, after which the filter and precipitate together are dried at 100° C. They are then allowed to cool, and small quantities of bisulphide of carbon added in three portions of about 2 or 3 cc., the bisulphide of carbon removed by the addition of æther, and the filter dried at 110° C. until it attains a constant weight. The difference between this weight and that of the filter as previously ascertained expresses

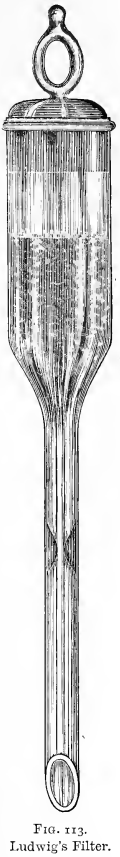


Fig. 113.
Ludwig's Filter.

the amount of uric acid in the quantity of urine taken. The dried filter containing the uric acid may be conveniently weighed by placing it in the scale upon a little triangular glass support of known weight and hollowed into a suitable angle, in which the thin end of the filter rests. The disturbing oscillation of the latter in the scale-pan may be prevented in this way.

It may be remarked that Ludwig's process has the advantage over the other methods referred to that by its means several observations can be made in one day.

A healthy adult excretes 0.2–1.0 grm. of uric acid with the urine in twenty-four hours. The quantity is increased in health by an abundant animal diet, and pathologically in fever, leukæmia (*Fleischer* and *Penzoldt*[295]), pernicious anæmia, and in diseases of the heart and lungs with obstructed respiration.[296]

The excretion of uric acid is diminished in a number of chronic affections, such as nephritis, gout (after the acute paroxysm), diabetes, and chronic arthritis. A diminution was also found by *v. Bamberger*[297] in a case of progressive muscular atrophy.

Finally, we sometimes meet with a condition in which, with emaciation and certain subjective symptoms, as hypochondriasis, &c., is associated an enormous increase in the elimination of uric acid as the only objective manifestation, and such cases undoubtedly constitute what is called the uric acid diathesis.

XV. Urea.—By far the greater part of the nitrogen taken in with the food is eliminated as urea. It must not, however, be forgotten that there are in the urine other nitrogenous compounds, such as uric and hippuric acids, amido- and other acids and ammonia salts, but the great bulk of nitrogenous waste is removed in the form of urea. Of this body 32–40 grms. are daily excreted by a healthy man, but its quantity varies considerably under physiological, and still more under morbid, conditions.

Amongst diseases, fever and diabetes are attended with increased elimination of urea. On the other hand, this is diminished in chronic affections accompanied by malnutrition and in diseases involving the proper structure of the liver, where it is elaborated (*Schröder*). Variations in the output of urea are an expression of changes in nitrogenous metabolism generally, and as such possess the highest clinical interest. It would be well if we could measure the quantity of that body in the urine at any given time, but the processes by which this can be done are not available for clinical purposes.

An approximate estimate of the quantity of urea excreted in the course of twenty-four hours may, however, be effected by *Hüfner's*[298] method. In this the urea is decomposed by means of alkaline hypo-

bromites, and the nitrogen given off as a gas is collected, whilst the carbonic acid combines with caustic soda present. The apparatus required is represented in the accompanying figure. It consists of a cylinder of stout glass of 100 cc. contents (B), expanding at its middle and connected below by means of a binder and tight-fitting tap with a smaller tube (A), also of glass, which holds about 5 cc. It is important

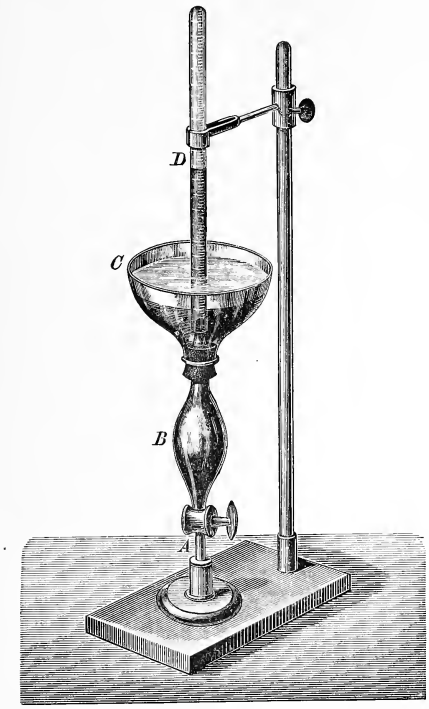


FIG. 114.—Hüfner's Apparatus.

that the capacity of the latter, which serves to receive the urine, together with that of the perforation in the tap, shall be accurately known. To this end the apparatus is carefully washed with water, rinsed out with alcohol, and dried. The tap is opened, and mercury poured into the lower vessel so as to overflow into the upper one. The tap is now closed, the overflow of mercury above it poured off, and the

contents of A removed and weighed in a vessel whose own weight is known. The result divided by the sp. gr. of mercury (13.59) gives the cubic contents of the tube A. This must be verified by repeating the process, and if the capacity ascertained should vary each time, the mean must be taken. It should be calculated to three places of decimals.

In cases where the method of weighing cannot be conveniently carried out, the capacity of the vessel destined to hold the urine may be ascertained with great accuracy thus:—This portion of the apparatus is filled with a watery solution of some aniline dye which is not taken up by chloroform, the apparatus washed out with chloroform and the tinted water together with the chloroform, in which little or none of the dye dissolves, is placed in an accurately graduated burette. The chloroform is allowed to settle and the quantity of the aniline fluid in the burette is read off. This process should be gone through at least three times, and the mean is taken of the ascertained results, which, however, usually agree well.

The remaining parts of the apparatus are a glass bowl (C) fitting by a caoutchouc stopper in its bottom upon the upper extremity of B, and a glass tube 30–40 cm. in length, 2 cm. wide, and accurately graduated in 0.2 cm. units of capacity.

A rough estimate of the proportion of urea is formed either by previous analysis, or better, by inference from the sp. gr. of the urine, and the latter diluted in such a way that a specimen shall contain not more than 1 per cent. urea. The vessel A, whose capacity is accurately known, is filled by means of a long funnel with urine, the well-greased tap closed, and the bulbous vessel B washed well with water, so as to remove all trace of urine from its surface. Another tube, about half a metre long, may be interposed between the upper end of B and the bowl (C), with the object of prolonging the contact between the column of urine and the hypobromite, and so securing its complete decomposition.

A fresh solution of hypobromite is then made in the following manner:—100 grms. of caustic soda are dissolved in 250 cc. of water, the mixture allowed to cool, and 25 cc. of bromine added. The solution must be freshly prepared for use, withheld from the light, and kept in a cool place. The concentrated fluid so prepared gives better results than the more diluted reagent formerly in use (*Pflüger* and *Schenck*).[299] With the vessel C in position, B is entirely filled with the above solution, and a concentrated solution of common salt is poured into C to a depth of 1 cm. The graduated tube D is likewise filled with the common salt solution, care being taken to exclude air-bubbles, and inverted in C over the tapering extremity of B, which projects into the solution of common salt contained in that vessel. It is fixed by a clamp in this position. Distilled water may be substituted for the salt solution.

The tap is now opened. The relatively heavier hypobromite solution sinks, and as it does so a rapid evolution of gas takes place and lasts for 15–20 minutes. The nitrogen formed collects in the graduated tube, and when it has ceased to be given off, the latter is closed with the thumb and, still inverted, placed in a cylinder of pure water. It is there held with a clamp, and is depressed as much as possible for a space of fifteen minutes. After that it is raised with wooden forceps until the fluid in the tube and that in the cylinder stand at the same level. The volume of contained gas is then read off, and the barometric pressure and temperature are noted at the same time.

From the volume of nitrogen obtained in this manner the weight in grammes of the urea taken may be deduced by the following formula:—

$$G = \frac{v\,(b - b')}{354.3 \cdot 760\,(1 + 0.00366t)}$$

Where

G = weight of urea in grammes.
v = volume of gas generated (in cc).
t = temperature.
b = barometric record.
b' = tension of water-vapour at temperature t.

The percentage of urea is expressed by the product of $G \times 100$ divided by the volume of urine analysed. The number 354.3 is substituted in the equation for 372.7, since it has been found that from 1 grm. of urea the total quantity of nitrogen, namely, 372.7 cc., is never obtained in this way, and that the number chosen more aptly represents the fact.

The value of b' will be found in Bunsen's tables, from which the following figures are extracted.[300] They express in millimetres the tension of water-vapour at the corresponding temperatures, which are those most commonly existent:—

10° C. . . 9.165	14° C. . . 11.908	18° C. . . 15.357	22° C. . . 19.659
11° C. . . 9.792	15° C. . . 12.699	19° C. . . 16.346	23° C. . . 20.888
12° C. . . 10.457	16° C. . . 13.536	20° C. . . 17.391	24° C. . . 22.184
13° C. . . 11.162	17° C. . . 14.421	21° C. . . 18.495	25° C. . . 23.550

To carry on such investigations uninterruptedly at least two sets of apparatus should be available. The researches of *Pflüger*[301] and his pupils have shown that the results obtained are not entirely accurate, but sufficiently approximate. The method has the advantage over others presently to be described, that it can be carried out quickly. Moreover, the object of a clinical investigation is less often to ascertain the precise quantity of urea than to determine its variations at

different times, and this purpose the method admirably fulfils. Recently a number of other apparatus of the same kind have been employed.[302] That of *Lange* seems specially serviceable.[303]

The quantitative estimation of urea may also be effected by Liebig's titration method, as modified by Pflüger. It will be found described at length in the systematic works of *Huppert*, *Hoppe-Seyler*, and *Leube-Salkowski*.[304]

To estimate the total quantity of nitrogen obtainable from the urinary products other methods are needed, as those of *Will-Varrentrapp* and *J. Kjeldahl*.[305]

The qualitative tests for urea are described in the chapter on Blood (p. 46). In connection with urine they possess but little practical interest.

XVI. Kreatinin.—In addition to the substances mentioned at p. 256 human urine contains certain other nitrogenous organic compounds, by means of which nitrogen is also eliminated from the system. Amongst these are betain, hypoxanthin (sarkin), xanthin, xanthokreatinin, kreatin and kreatinin. The separation of these substances is, with the exception of the last, without clinical significance at present. The interest attaching to the separation of kreatinin is of a very subordinate kind, and it will suffice to state a few facts concerning its production, detection, and clinical import. For information about the other substances named the reader may consult the books of reference already referred to.

The formation of kreatinin has been shown to be intimately associated with the decomposition of muscle-substance, and the quantity produced is in direct relation to the amount of flesh-meat consumed as food, and, under certain circumstances, to the muscle-waste within the body. Under these circumstances muscle contains kreatin, which is changed into kreatinin as it passes through the body.

This is to be borne in mind when a clinical inference is drawn from the observation of an increase or diminution of the quantity of kreatinin secreted. Hitherto such inferences as were possible have had but a limited application to the purposes of diagnosis. They are insufficiently based, and rest for the most part on the experience of individual cases. According to *Neubauer*,[306] the quantity excreted by a healthy man is about 1 grm. According to *Pouchet*,[307] it is 1 grm. in the case of the male, 0.75 grm. in that of the female, while kreatinin is altogether absent from the urine of sucklings. This point, however, is contested by *Grocco*.[308]

An increase in the output of kreatinin has been observed in acute diseases of all kinds while attended with fever, and in diabetes (*Senator*[309]), and a diminution in chronic nephritis and diabetes insipidus

in convalescence after acute diseases, in chlorosis, anæmia, tuberculosis, and marasmus.[310]

Kreatinin is a base which forms highly characteristic compounds with acids, such as phosphotungstic and phosphomolybdic, hydrochloric and sulphuric, and with salts of the heavy metals.

Qualitative Tests.—Kreatinin may be detected directly in the urine by the methods of *Weyl*[311] and *Jaffé*.[312]

Weyl's Test.—For the application of this test the urine should be freed from acetone by distillation (p. 249). A freshly prepared and very dilute solution of nitroprusside of soda and caustic potash is added. If kreatinin be present, the fluid will take a beautiful red colour (like that produced in *Legal's* test for acetone), which soon disappears, and does not return with the further addition of acetic acid.

Jaffé's Test.—A fairly concentrated solution of picric acid and a little caustic potash are added to the urine. If the fluid be heated, the presence of kreatinin will be shown by the appearance of a beautiful red coloration. Acetone and grape-sugar yield a similar reaction. Picric acid with caustic potash alone gives a slight red colour.

Quantitative Estimation.—Kreatinin forms with zinc chloride a double salt of slight solubility, and this property is made the basis of the quantitative method, which was first devised by *Neubauer*[313] and modified by *Salkowski*.[314]

Two hundred cc. of urine are mixed with a little milk of lime until the fluid has an alkaline reaction. This is to precipitate phosphoric acid. A solution of calcium chloride is added until a precipitate ceases to form. The fluid is allowed to stand for half an hour, after which the precipitate is filtered off and repeatedly washed with water, and the filtrate and washings (acidulated with a little sulphuric acid) are evaporated on the water-bath to a syrupy consistence. To the residue is added 50–100 cc. of 78 per cent. alcohol; the mixture is well stirred and allowed to stand in the cold for several (6–8) hours; then it is filtered, and to the filtrate (which, if alkaline, must be rendered acid with acetic acid) 10–15 drops of an alcoholic solution of zinc chloride are added. The latter is prepared by adding alcohol to a concentrated solution of zinc chloride until a density of 1.2 is attained. After the lapse of two or three days the precipitate is brought upon a filter of known weight, and the filtrate as it passes through is constantly returned to wash the vessel which contained the precipitate. When all the precipitate has been brought upon the filter in this way, it is washed with 90 per cent. alcohol until the filtrate shows but slight opalescence with silver nitrate; after which it is dried to a constant weight at 100° C. One grm. kreatinin-zinc chloride corresponds to 0.6242 grm. kreatinin. The amount of kreatinin in the quantity of

urine taken away may therefore be known by multiplying the quantity of kreatinin-zinc chloride obtained by 0.6242.

Kreatin, which is closely related to kreatinin, does not occur as such in the urine, but it is readily formed from kreatinin in alkaline fluids. Hence it follows that alkaline urine should not be employed clinically for the quantitative estimation of kreatinin.

In addition to kreatin a number of basic substances have recently been isolated from urine by precipitation with phosphotungstic acid (*Thudichum*[315]), such as urochrome, urotheobromin, omichol, and reducin. The physiological action of these substances is not yet known.

Salomon[316] has proved that hypoxanthin is a normal constituent of urine. Future researches will show in what relation it stands to certain diseases, or to the putrefaction bases presently to be described.

XVII. Ptomaines (Putrefaction Bases) in the Urine.—It would appear from the investigations of *Pouchet*[317] that healthy urine contains traces of certain toxic substances of an alkaloid character, and according to the researches of *Bouchard*,[318] *Lépine*,[319] and *Guerin*,[319] these bodies are more abundant under morbid conditions. They were found by *A. Villiers*[320] as an invariable manifestation in measles, diphtheria, and pneumonia; and in the urine of cholera *A. G. Pouchet*[321] discovered an alkaloid which was not identical with that observed by him in the fæces of the same disease (see p. 164). *Feltz*[322] found similar bodies in the urine of cancer patients. *Bouchard*[323] discovered that human urine acted as a poison when injected within the veins of animals (rabbits), and he referred the effects to various substances, among which were animal alkaloids. For the detection of the urinary alkaloids the following method has been adopted by *Tanret*, *Bouchardat*, and *Cardier*[324]:—

To the urine which has been acidulated with acetic acid a solution of the iodide of mercury and potassium is added. The precipitate, which contains the alkaloids, is readily distinguished from those of other substances, as albumin, mucin, and uric acid, obtained with the same reagent, by its solubility in alcohol at a warm heat.

Ch. Bouchard rendered the urine alkaline with caustic soda, and derived a poisonous body as an æthereal extract.

Pouchet fixed the alkaloid by combination with tannic acid, and subsequently precipitated it with oxide of lead from alcoholic solution.

The methods adopted by the other observers mentioned above differed much as to details. They will be found described at length in the original communications. That of *Brieger* (p. 119) serves best for the detection of animal alkaloids in the urine. In some cases, however, it is important that the urine be previously concentrated in vacuo. Should

no result be obtained with this, *Gautier's* [325] method may next be tried. Finally, the Stas-Otto method may be applied to the same purpose. (See p. 118).

The diamines of the urine may be precipitated as benzoyl compounds, and best by the action of benzoyl chloride and caustic potash. *Baumann* and *Udransky* [326] succeeded in separating several basic derivatives, amongst them cadaverin (pentamethylendiamine), putrescin (tetramethyl diamine), and a small quantity of a third diamine, from the urine of a patient with cystinuria and vesical catarrh. Normal urine was found to be free from these bodies. The author has been for some time engaged in observing the occurrence of similar poisonous bases in the urine of health and disease, and he has found that while normal urine and that of some diseases hold such only in very small quantity, in certain morbid states their amount is very considerable. He would venture to make some suggestions for the benefit of those who are engaged with similar researches. In the first place, it would be well to follow the example of Brieger, Baumann, and v. Udransky, and withhold the name of alkaloids from the bodies (diamines) alluded to, which are derived from the system under morbid conditions, because all that have been recognised as yet are simply diamines, and because none yet examined exhibit the characteristic property of alkaloids, namely, the pyridin radicle. Next, it would be desirable to discriminate between the physiological bases of the urine (kreatinin, reducin, &c.), which belong normally to the fluid, and those which are associated only with certain diseased states. It is not intended to imply that the physiological bases cannot under any circumstances give rise to the symptoms of disease or of poisoning. (See below.) Experience is not wanting to make it seem in the highest degree probable that the retention, and still more the increased formation, of such physiological products in certain diseases may induce symptoms of the gravest character, and greatly imperil the life of the patient.

Again, it would appear that in certain acute affections specific substances of a toxic character, not observable in normal urine, may be excreted with that fluid. Undoubtedly the matter is somewhat obscure. The author's views may be stated as follows. It is possible to distinguish :—

(1.) Clinical (morbid) symptoms depending upon the retention of the physiological bases (and under this heading would come uræmia) and certain of the symptoms of obstruction (*retention-toxicosis*).

(2.) Clinical symptoms, referable to the presence of basic products, which are formed in the system (blood, &c.) in disease and eliminated with the urine (*noso-toxicosis*).

(3.) Clinical symptoms which are caused by the formation of toxic

basic substances from morbid matter, such as pathological fluids lodged in certain parts of the system. Such bases are absorbed, and then give rise to manifestations of severe poisoning. Under this heading would come the collective symptoms of ammoniæmia (see p. 51), and others which follow the absorption of gangrenous pus (*auto-toxicosis*).

(4.) Clinical symptoms, and consequently morbid types, induced by the action of toxic bases taken into the system with the food, such as the poison of sausages and cheese (*exogenic toxicosis*).

These distinctions are based partly upon clinical observation and partly upon experiments on animals. They will serve as a scheme for the further elucidation of this very important subject.

If we have dwelt at some length on the result of observations which are not yet completed, we have done so because we believe that the careful investigation of the urine in this direction will throw light upon the nature of some diseases which are at present not sufficiently understood.

XVIII. The Ferments of the Urine.—The appearance in the urine of a body resembling pepsin was long ago established by *v. Brücke*.[327] *Sahli*, *Leo*, *Gehrig*, *Stadelmann*, and *Patella*[328] made similar experiments and confirmed the presence of pepsin in the urine. It has been asserted that trypsin is also an occasional constituent of that fluid, though some observers, *Leo*, *Stadelmann*, and *Grützner*[329] have failed to find it.

With reference to the pepsin ferment of the urine, clinical interest attaches to it from the fact that it is absent in typhoid and carcinoma of the stomach, and according to some observers (*Mya*, *Belfanti*[330]) in nephritis.

For the detection of pepsin Sahli's method (adapted from those of v. Wittich and Grützner) may be employed. It is founded upon the property which, as v. Wittich originally observed, blood fibrin possesses of readily absorbing that body from solutions. To this end a little pure fibrin is placed in the urine and allowed to rest there several hours. It is then removed, placed in dilute hydrochloric acid (.2 per cent.), and the mixture kept at a temperature of 30–40° C. Any pepsin present is precipitated on the fibrin, and the latter is slowly digested in the acid fluid.

Diastatic ferment also is said by *Hovoltschiner* to occur in urine.[331] The researches of *Breusing*[332] and observations made by the author tend to show that the ferment in question is in many cases not diastase, but an amylolytic substance. At the same time it must be stated that the author has frequently determined the presence of diastase, by the usual methods, both in health and disease. So, too, *Leo*[333] has found diastase both in healthy and in morbid urine.

Milk-curdling ferment is occasionally present in the urine (*Hovoltschiner*,[334] *Boas*[335]).

As to whether the urine contains a ferment capable of decomposing urea into carbonic acid and ammonia, opinions differ. On the one hand, *Musculus*[336] believes that he has isolated such a ferment, but *Leube*[337] has sought for it in vain in urine which was actually undergoing ammoniacal fermentation.

B. Inorganic Substances.—The inorganic constituents of the urine are for the most part salts of hydrochloric, sulphuric, and phosphoric acids, to which must be added carbonates, silicates, nitrates and nitrites, and sulphuretted hydrogen.

1. Chlorides.—The chlorides of sodium, potassium, ammonium, and magnesium are found in the urine, and of these we are chiefly concerned with chloride of sodium. Of this salt 10–15 grms. are voided by a healthy man in twenty-four hours; but its quantity is greatly influenced, even in disease, by the supply of salt taken as food.

It is increased by an abundant diet and as a consequence of conditions which determine the retention of chlorides within the system; and diminished in fevers, and notably in croupous pneumonia.[338] The elimination of chloride of sodium has also been observed to be less in cases of chronic nephritis, and sometimes in certain diseases of the stomach (*Gluzinski*).[339]

Detection of Chlorides.—The urine is treated with nitric acid, and a solution of nitrate of silver added. A caseous precipitate soluble in ammonia, insoluble in nitric acid, shows the presence of chlorides.

Quantitative Estimation of Chlorides.—Mohr's method is to treat the urine with chromate of potash, and gradually add nitrate of silver, when all the chlorine combines with the silver to form silver chloride, and the occurrence of a red precipitate (chromate of silver) marks the end of the reaction. The details of the process will be found in works on urinary chemistry. *Salkowski's*[340] modification of *Volhard's*[341] method is to be preferred.

When to a solution of nitrate of silver acidulated with nitric acid is added some of a solution of sulpho-cyanide of ammonium, a curdy white precipitate forms, and this, like chloride of silver, is insoluble in nitric acid, soluble in ammonia. If the fluid also contains a ferric salt, a blood-red colour (ferrocyanide) forms at the moment when the last of the silver is precipitated. If now the sulpho-cyanide of ammonium solution is of a known degree of concentration, it is possible to determine the quantity of silver present by noting the point at which the red coloration takes place. To apply this principle for gauging chlorides in solution to the fluid containing them a silver solution of a definite degree of concentration is added in excess, and that portion of it which

is not precipitated as chloride of silver is measured in the manner indicated above.

The following solutions are needed in the process:—

i. *Pure nitric acid* of 1.2 sp. gr.

ii. *Concentrated solution of double sulphate of iron and ammonia free from chlorine.* It is necessary that this be free from chlorine, and if not already so, it must be purified by crystallisation.

iii. *Nitrate of silver solution of definite strength.* The chemically pure crystalline salt is dissolved in water in the proportion of 29.075 grms. to the litre. A cc. of this solution corresponds to 0.01 grm. of chloride of sodium.

iv. *Sulpho-cyanide of ammonium solution.* This should be of such a constitution [341] that 25 cc. shall correspond to 10 cc. of the silver solution. For this purpose 6.5–7 grms. of sulpho-cyanide of ammonium may be dissolved in water, and more water added to 400 cc. A burette is filled with the solution so prepared.

Titration with the sulpho-cyanide of ammonium solution is effected thus:—Ten cc. of the silver solution (iii.) is placed in a flask and diluted with water to 100 cc.; 4 cc. of nitric acid (i.) are next added, and after that 5 cc. of the double sulphate of iron and ammonia (ii.). The mixture is well shaken up, and sulpho-cyanide of ammonium solution from the burette is carefully added until a slight but permanent red colouration appears. The process is repeated several times, the quantity of the reagent employed in each case being noted and the mean taken.

In accordance with the result obtained, the sulpho-cyanide of ammonium solution is diluted to such a point that 25 cc. shall correspond to 10 cc. of the silver solution. Thus, if the terminal reaction (red colour) occurs after the addition of 22 cc., the following formula may be applied to determine the volume to which a litre of the solution must be diluted:—$22 : 25 = 1000 : x$, and $x = 1136.3$. To the litre of sulpho-cyanide of ammonium, therefore, 136.3 cc. must be added in order that 25 cc. shall correspond to 10 cc. of the silver solution (iii.).

The remainder of the process is as follows:—10 cc. of urine are measured out with a pipette and placed in a graduated flask of 100 cc. capacity; 50 cc. of water are added, and then successively 4 cc. of the nitric acid (i.) and 15 cc. of the silver solution (iii.). The flask is closed with a glass stopper and well shaken up until a precipitate ceases to form and the fluid tends to clear; the flask is then filled to the mark 100; its contents passed through a dry paper filter and received into a dried measure cylinder or a flask holding 80 cc. The 80 cc. of fluid thus obtained are poured into a larger flask of some 250 cc. capacity; 5 cc. of the double sulphate of iron and ammonia (ii.) are added, and the sulpho-

cyanide of ammonium solution (iv.), prepared in the manner already indicated, is gradually supplied from a burette, until the terminal reaction is shown on shaking the fluid by a faint but abiding red colouration. The quantity of the sulpho-cyanide of ammonium used to effect this is now read off. Experience has shown that 15 cc. of the silver solution is more than sufficient to precipitate all the chlorine from urine strongly acidulated with nitric acid, and that an excess of silver nitrate remains in solution. The excess of silver may be measured volumetrically by means of the sulpho-cyanide of ammonium solution, and the quantity of chlorine in the urine calculated from the difference.

The quantity of chloride of sodium in grammes in one litre of the urine may be ascertained by the following formula :—

$$x = [37.5 - \tfrac{5}{4} R] \tfrac{4}{10}$$

Where

x = the quantity of NaCl in a litre of urine in grms.
R = the quantity of sulpho-cyanide solution used in cc.

The formula is derived thus :—

Ten cc. of the silver solution correspond to 25 cc. of the sulpho-cyanide of ammonium, and consequently 15 cc. of the former to 37.5 cc. of the latter. For 100 cc. of the fluid examined, therefore, are needed 37.5 cc. of the sulpho-cyanide solution less by five-fourths of the total quantity of the latter employed. Since 80 cc. of the titration fluid, *i.e.*, sulpho-cyanide, was used, consequently 100 cc., being the volume of the original fluid, will require five-fourths of the quantity read off (on the burette).

Now 25 cc. of the sulpho-cyanide correspond to 10 cc. of the silver solution, and therefore 1 cc. of the former to 0.4 cc. of the latter.

One cc. silver solution corresponds to 0.01 grm. sodium chloride ; 0.4 cc. silver solution corresponds to 0.004 grm. sodium chloride.

Therefore, to obtain the quantity of chloride in the volume (10 cc.) of urine tested, the expression $[37.5 - \frac{5}{4} R]$ must be multiplied by 0.004, or by $0.4 = \frac{4}{10}$ for 1000 cc. (litre) of the urine.

2. Sulphates.—Sulphuric acid is present in the urine both as simple (preformed) sulphuric acid and as æther-sulphuric (compound sulphuric) acid (see p. 241). The combinations of the latter acid have been already spoken of. Further, the urine contains sulphur as sulpho-cyanides and hypo-sulphites (thio-sulphates [342]) (see also p. 271).

The quantity of sulphuric acid excreted in twenty-four hours by a healthy adult under ordinary conditions of diet is about 2 grms., of which 0.1 grm. is in the form of æther-sulphuric acid compounds.

Sodium, potassium, magnesium, and calcium sulphates are found in

the urine (see pp. 108, 202.) But little clinical significance attaches to a general increase or diminution in the output of sulphuric acid in disease. Of far greater import are changes of the relative quantities of simple and of æther-sulphuric acids (see p. 246).

Thus a urine rich in indigo compounds contains little of the preformed sulphuric acid, and in carbolic acid poisoning this may entirely disappear.

Detection of Simple Sulphuric Acid.—The urine is filtered if turbid, acidified with acetic acid, and solution of chloride of barium added. A fine precipitate, barium sulphate, forms. This reaction never fails with the normal fluid.

Estimation of Simple Sulphuric Acid.—This may be effected by determining first the total quantity of sulphuric acid present, and then that of æther-sulphuric acid, according to the methods indicated at p. 245. The difference will be the quantity of free sulphuric acid sought.

Estimation of the Total Quantity of Sulphur.—Where it is of consequence to ascertain the total quantity of sulphur in the urine, the best plan is to evaporate the alkaline urine, either the whole or a known proportion of it, on the water-bath, then to fuse the incinerated residue with saltpetre and soda,[343] to extract the fused mass with boiling water, and to proceed further as directed in the process for determining the total quantity of sulphuric acid on p. 246. The extract is treated with barium chloride, and the sulphur estimated as barium sulphate.

3. Phosphates.—The phosphoric acid of the urine is combined partly with sodium, potassium, and ammonium, and partly with lime and magnesia. Being a tribasic acid, it forms three classes of salts—acid, neutral, and basic. Of these, the acid phosphates of alkalies and alkaline earths and the neutral and basic phosphates of the alkalies are soluble in the urinary fluid. The neutral phosphates of the alkaline earths are but little soluble therein, and their basic phosphates still less so.

Upon this fact depends the deposition of phosphatic sediment when the urine is boiled. The acid and neutral phosphates of the alkaline earths are changed in the process into the insoluble basic salts. The phosphorus salts occur partly in solution and partly as a crystalline deposit (see pp. 197, 202).

Two to three grms. of phosphoric acid is excreted in twenty-four hours.

It appears, especially from the researches of French authors,[344] that in certain morbid states the phosphatic constituent undergoes notable increase, and that consequently a condition of phosphaturia, analogous to that of oxaluria, may properly be spoken of; and further, that this condition may take the place of glycosuria in diabetes. The subject, however, is still under discussion. A diminished elimination of

phosphates was observed by *Stokvis*[345] in arthritis, and *v. Jaksch*, unlike other observers, has found that in some, though not all cases of lobar pneumonia amongst children, the quantity of phosphoric acid eliminated during the continuance of fever was increased as compared with the non-febrile period.

A phosphatic sediment does not imply phosphaturia. The diagnosis of this condition can be safely based only upon the quantitative estimation of phosphoric acid, and this is effected best by *Neubauer's*[346] method of titration with a solution of uranium oxide (see below).

Detection of Phosphates.—The urine is treated with caustic potash and heated. The phosphates are precipitated as earthy phosphates. By the addition of ammonia they may be precipitated without heat.

To detect the presence of phosphoric acid in combination with alkalies, the urine is treated with ammonia and filtered, and to the filtrate an ammoniacal solution of magnesia (a mixture of sulphates of magnesia and ammonia) is added, whereby the phosphates are precipitated as triple phosphate.

Another method is to treat the filtrate (*vide supra*) with acetic acid, when the further addition of uranium solution yields a yellowish-white precipitate.

The same filtrate with perchloride of iron solution gives a white precipitate, which becomes yellow on the addition of more of the perchloride.

Estimation of Phosphoric Acid.—To urine which contains the phosphates as acid phosphates uranium acetate or nitrate is added in solution until an excess of the reagent first becomes appreciable. If the nitrate be used, free nitric acid forms, and causes a part of the precipitated uranium phosphates to redissolve. To prevent this in practice a little sodium acetate is added to the urine before titration with uranium nitrate. As an indicator a solution of ferro-cyanide of potassium is employed. This yields an intensely brown precipitate in presence even of a mere trace of a uranium salt.

This test, however, is less sensitive in presence of acetate of soda than in simple watery solutions. Hence it is necessary to use a definite quantity of the salt, and to take care that the proportion is maintained in preparing the titration fluid.

The solutions required for the process are :—

i. *Solution of Acetate of Soda.*—A hundred grms. of acetate of soda are dissolved in 800 cc. of water, 100 cc. of a 30 per cent. solution of acetic acid added, and the mixture made up to a litre. Five cc. are employed with 50 cc. of urine.

ii. *Solution of Ferro-cyanide of Potassium.*—Twenty-five grms. of

ferro-cyanide of potassium are dissolved in 250 grms. water, and the preparation, which should exhibit only a faintly yellow tint, is to be kept in a dark place.

iii. *Solution of Uranium Oxide.*—About 20.3 grms. of commercial uranium oxide purified and well dried is dissolved in pure acetic acid, or in the smallest possible quantity of nitric acid, and the preparation made up to a litre. Of the mixture 1 cc. indicates 5 mgrms. of P_2O_5.

iv. *A Solution containing a Definite Quantity of Phosphoric Acid.*—Fifty cc. should contain precisely 0.1 grm. P_2O_5. The preparation is made by dissolving 10.085 grms. of neutral phosphate of soda in a litre of water. The commercial salt should be crystallised from solution to obtain it free from chlorine, so that no precipitate forms with nitrate of silver and nitric acid. The crystals are then placed on paper in a funnel, the neck of which is stopped with glass wool and allowed to dry there until the mother liquid is no longer found to adhere to them. A known weight is then taken and rubbed up in a mortar and a portion of the powder submitted to a gentle heat in a platinum crucible, and finally incinerated. 266 grms. of sodium-pyrophosphate ($Na_4P_2O_7$) correspond to 716 grms. $Na_2HPO_4 + 12H_2O$. Consequently that quantity of the dried crystals which, when incinerated, yields 266 grms. corresponds to 716 grms. of pure phosphate of soda.

v. *Titration Process.*—Fifty cc. of the phosphoric acid solution (iv.) are measured into a flask, 5 cc. of the solution of acetate of soda (i.) are added, the mixture boiled, and the uranium solution (iii.) gradually supplied until a precipitate ceases to form. In the process a high temperature should be maintained, to promote the formation of uranium phosphates.

When the precipitate no longer increases, the terminal reaction is tested for. To this end the fluid is again heated. A drop is placed in a white porcelain capsule, and a drop of solution of ferro-cyanide of potassium (ii.) added. Should no brown colouration take place, 0.5 cc. of the uranium solution is supplied, and the terminal reaction again sought. When this is obtained the process is repeated, with the addition at the outset of a quantity of uranium solution, which shall be less by 0.5 cc. than the total supply on the former occasion; the mixture is again boiled, and uranium solution gradually added—not more than 0.1 cc. at the time—until the first indication of a reddish-brown colour appears. Should it happen that the fluid cools during the process, it must be boiled anew.

The uranium solution is now diluted, according to the quantity found to be necessary, as above, in such proportion that 20 cc. shall just suffice for the titration of 50 cc. of the phosphoric acid solution.

Now, 50 cc. of the phosphoric acid solution represent 0.1 grm. P_2O_5,

and consequently 20 cc. of the diluted uranium solution also correspond to 0.1 grm. P_2O_5.

The titration process is repeated with the urine in precisely the same manner as before: 50 cc. are taken, 5 cc. acetate of soda added, and the mixture heated and the terminal reaction sought.

Every cubic centimetre of the uranium oxide solution employed in titration represents 5 mgrms. P_2O_5. Hence the phosphoric acid contained in 50 cc. of urine may be calculated by multiplying the number of cubic centimetres of uranium oxide solution used by 0.005. The result is the quantity of phosphoric acid in grammes contained in 50 cc. of urine. It is advisable in each case to make two such investigations and to take the mean of their results.

4. Carbonates.—The carbonates of lime, magnesia, and ammonia are sometimes present in the urine. The latter salt, however, is found in large quantity only as a result of alkaline decomposition. *Heintz* has proved that ammonium salts may be detected in every specimen of urine, whether decomposed or not. They may be shown best by the addition of milk of lime in a test-tube, when ammonia is given off, and if a piece of red litmus paper be moistened and held over the mouth of the test-tube, it becomes blue.

Test.—The presence of carbonates in the urine is shown by the evolution of a colourless gas on the addition of acid, and this gas will render baryta water turbid.[347]

5. Nitrates and Nitrites.—Nitrates and nitrites occur in the urine (*Schönbein*).[348] Nitric acid is thought to be derived from the water and food ingested (*Röhmann*).[349]

Nitrites occur only in decomposing urine, and are derived from the reduction of nitrates in urinary fermentation.

Tests for Nitrites:—

(*a.*) Solution of iodide of starch paste in presence of dilute sulphuric acid.

(*b.*) Metadiamido-benzol (see p. 56) is coloured a deep yellow by nitrites.

Amongst the inorganic constituents of the urine there remain to be mentioned silicic acid and salts of iron, which are sparingly represented.

Strümpell[350] found hyposulphurous acid in a case of typhoid. The urine became turbid from the separation of sulphur on the addition of hydrochloric acid.

6. Sulphuretted Hydrogen (Hydrothionuria).—Sulphuretted hydrogen is rarely present in the urine, but it can always be obtained from it by heating with mineral acids.[351] It has been ascertained (*Betz, Senator, Ottavio Stefano*[352]) that when retained in the system

in considerable quantity it may produce toxic effects (auto-intoxication). In the great majority of cases, according to *Müller*[353] and others, hydrothionuria is due to a sulphuretted hydrogen fermentation of the urine, caused by the action of certain micro-organisms.

This gas is sometimes derived from the alimentary canal, and its presence depends upon an abnormal communication between the urinary passages and the gut. *Betz* further maintains that it may pass by endosmosis from the intestine into the urine; and it would appear also that the gas may be absorbed from the intestine by the blood, and so find its way into the urine. This, according to Müller, is a rare event, and happens only when the quantity of sulphuretted hydrogen is so great as to give rise to symptoms of general poisoning.

Tests.—The urine, which should be acid, is placed in a flask, which is closed by a tight-fitting cork. From the latter depends a strip of blotting-paper soaked in sugar of lead and caustic soda. If sulphuretted hydrogen be present, the paper turns black.

Fr. Müller recommends the following plan:—A current of air is passed through the urine, and directed by means of a fine-pointed glass tube upon a strip of blotting-paper soaked in alkaline sugar of lead solution. If sulphuretted hydrogen be present, the paper is blackened. *Emil Fischer's*[354] test is also applicable to the urine. Some particles of p-amido-dimethylanilin are added to a few cc. of water, and a little concentrated sulphuric acid and one or two drops of a yellow solution of perchloride of iron supplied. The reagent is poured on the surface of the urine to be tested, when, if sulphuretted hydrogen be present, a blue ring (methylene-blue) forms at the place of contact of the two fluids. This ring often takes some minutes to develop.

7. Peroxide of Hydrogen.—This body was first observed in the urine by *Schönbein*.[355] Its presence there has no pathological import.

Test.—Dilute solution of indigo is bleached by peroxide of hydrogen in presence of sulphate of iron solution.[356] Tetra-paper (see p. 102) immersed in the fluid will show the presence of ozone by taking a blue colour.

8. Gases of the Urine.—The urine contains a small proportion of gases, which may be withdrawn from it by the air-pump. They are chiefly carbonic acid, oxygen, and nitrogen.[357]

IV. CHARACTERS OF THE URINE IN DISEASE.

I. The Urine in Febrile States.—In fever the urine is diminished in quantity, acid, deeply coloured, and of high sp. gr. On standing, it often deposits an abundant sediment of urates. Microscopically it exhibits a profusion of crystals of uric acid and urates, and a few

hyaline casts, with scattered leucocytes, renal epithelium, or fungi on their surface. It commonly contains a small quantity of albumin (febrile albuminuria) or acetone in variable proportion. Diacetic acid may be present when the disease is of an acute infectious character, or when its subject is a child. In the first case it betokens great danger; not so in the latter. [See Appendix XXII.]

The presence of peptone (p. 217), with or without serum-albumin and in conjunction with the clinical symptoms of a puerperal or hæmatogenic origin, indicates the formation and absorption of pus within the system.

According to *Ehrlich*,[358] it is characteristic of the urine in typhoid, measles, and acute tuberculosis to yield a deep-red colour with diazo-benzol-sulphonic acid.

Authorities differ much as to the diagnostic value of this reaction. On the one hand, the opinion of *Ehrlich* is supported by *E. B. Goldschmidt*,[359] while *Penzoldt*[360] and *Petri*[361] dissent from it. *Ehrlich* obtains the reaction, not with dia-benzol-sulphonic acid itself, but with sulphanilic acid. Fifty cc. hydrochloric acid are made up to 1000 cc. with water and sulphanilic acid added to saturation. To 200 cc. of the mixture 5 cc. of a $\frac{1}{2}$ per cent. solution of sodium nitrite are added, and the resulting fluid is added to the urine in equal parts. *Ehrlich*[362] has recently recommended that five to six times the volume of absolute alcohol should be added to the fluid to be tested, and the reagent, prepared as above, then added drop by drop to the filtrate. Normal urine gives a yellow colour, while the urine of fever patients, &c., turns scarlet.

It will be seen from this brief statement that a careful analysis of the urine will serve to make evident the details of acute processes earlier and more readily than the methods formerly at our disposal. Other lines of investigation are appropriate to certain acute affections; thus, for instance, in pneumonia the presence of chlorides will be sought.

II. The Urine in Disorders of the Circulation (Congestion).—Under such conditions the urine in its physical characters closely resembles that of fever. It is diminished in quantity, of acid reaction, and high sp. gr. (1.025–1.035). It commonly deposits urates.

Chemically it may be distinguished from fever urine by—

(1.) The absence of acetone and diacetic acid.

(2.) The presence of albumin in greater quantity.

Microscopically, and especially when the congestion is chronic, the urine exhibits some leucocytes and altered red blood-corpuscles, often also hyaline casts, and cylindrical aggregations of urates (see p. 180, fig. 75), waxy and a few granular casts and renal epithelium. Such constituents indicate secondary changes in the kidney of a chronic inflammatory character.

III. The Urine in Diseases of the Urinary Organs.

1. Renal Affections.

(*a.*) **Acute Nephritis.**—In this disease the urine is at first diminished in quantity—500–800 cc. or less being passed in twenty-four hours—of acid reaction, and high sp. gr. (1.015–1.025). The sp. gr. rarely attains to so great a height as in the urine of congestion. It ranges in colour from blood-red to that of a watery extract of meat, and the presence of blood-pigment in considerable quantity may be determined by *Heller's* test or by spectroscopic examination. In the latter case, if the urine be fresh, the characteristic bands of methæmoglobin may be visible.

Chemical analysis shows large quantities of albumin.

Microscopical investigation of the sediment affords the clue to the condition. It exhibits—

(1.) Red blood-corpuscles in variable proportion. These are for the most part altered, and present the appearance of washed-out discs (phantom corpuscles).

(2.) Some leucocytes. These are always less numerous than the phantom cells just mentioned.

(3.) Epithelium. Chiefly small polyhedral uninuclear cells from the urinary tubules, with a few others derived from the renal pelves and bladder.

(4.) Casts. These are (*a*) formed of blood-corpuscles; (*b*) formed of leucocytes; (*c*) formed of renal epithelium; (*d*) hyaline, more or less thickly beset with epithelial cells and red or white blood-corpuscles.

Such are the microscopical constituents of the sediment at the outset of an acute nephritis, as in the first and second days of the nephritis of scarlatina and erysipelas. They alter their character as the disease progresses, and after the lapse of a few days, and side by side with those described, appear the metamorphosed casts, granular and waxy, &c.

When in the course of chronic nephritis an acute exacerbation takes place, the urine possesses a similar character to that described above. But, as before, the description applies with full force only to the earlier period of the attack. If death from uræmia or œdema of the lungs does not ensue, the physical characters of the urine tend gradually to return to those of health. It is more abundant, the contained blood grows less, and then a light flesh-water tint alone declares the existence of acute nephritis. Albuminuria becomes less marked, and finally ceases with the approach of health. The other signs, recognisable only by means of the microscope, disappear with, or shortly after, the cessation of the albuminuria.

(*b.*) **Chronic Nephritis.**—The urine is normal in quantity, or some-

what lessened (1200–1500 cc. daily), acid, and of normal sp. gr. It usually contains a very considerable proportion of albumin. Microscopically the sediment is very variable in character, but renal epithelium is never absent, and the cells are often fatty. There are metamorphosed casts of different kinds, especially granular casts, and hyaline casts covered with blood-corpuscles or renal epithelium (p. 186). These are of special importance in diagnosis.

The occurrence of casts composed of fatty matter or overlaid with fat-crystals indicates advanced fatty degeneration of the renal tissue (p. 185).

It occasionally happens that, with all the symptoms of chronic nephritis present, no trace of casts or epithelium can be found in the urine. This occurs most often in cases of long-standing and running a very chronic course.

(*c.*) **Contracted Kidney.**—The quantity of urine is very greatly increased—4000 to 5000 cc. being passed in the twenty-four hours. Its reaction is acid, and sp. gr. low (1.008–1.012 and less). In this respect, however, exceptions are not uncommon. The author has met with cases in which the quantity of the excretion was diminished and the sp. gr. proportionally increased. It is pale in colour, and contains but little albumin, sometimes only a mere trace. The sediment is usually scanty, and, when examined with the microscope, exhibits generally but a few hyaline and some granular casts.

It is to be observed that even those cases in which only a trace of albumin is to be found (small red kidney of *Ribbert*) often run a particularly unfavourable course.

(*d.*) **Amyloid Kidney.**—In this condition the urine presents characters which are for the most part similar to those of contracted kidney, that is to say, it is increased (though sometimes normal) in quantity, of acid reaction, and low sp. gr. As a rule, however, it contains more albumin.

Microscopical examination of the sediment shows hyaline casts in comparative abundance and some epithelium. The microscopical appearances, however, are subject to much variety, and the author has observed cases which were hardly to be distinguished from those of chronic nephritis. The amyloid reaction (iodo-potassic-iodide and sulphuric acid, &c.) with the casts cannot be depended upon, since, on the one hand, it is often obtained when the *post-mortem* appearances show no amyloid degeneration of the kidneys, and, on the other, it is absent in many cases where the symptoms (enlarged spleen and liver, &c.) point to this condition.

(*e.*) **Uræmia.**—The urine in cases where the symptoms of uræmia supervene is always rich in albumin, and microscopically resembles

that of nephritis. It is scanty in a degree that sometimes amounts to anuria, and even when greatly reduced in quantity, we often find that its sp. gr. is still below the healthy standard. Again, *the quantity excreted may remain unaltered, but the sp. gr. in such cases is always greatly reduced.*

When the urine is of this character, it would seem to hold poisonous bases in smaller quantity than ordinarily.[363]

What has been said here applies only to *typical forms* of renal disease. The appearances are modified according to the various anatomical perversions of the kidney structure upon which they depend.

Investigations which the author has made as to the character of the urine of children suffering from nephritis show that the important constituents of the excretion, urea, uric acid, sulphuric and phosphoric acid, are constantly diminished in quantity.

2. Pyelitis Calculosa.—The urine passed during the paroxysms of this affection is diminished in quantity, contains mucin in abundance, blood and pus in variable proportions, and concrete masses of uric acid or urates. After the paroxysms the urine is passed in great abundance. It is then pale in colour and of low sp. gr., and exhibits probably a flocculent precipitate of mucin. Persistent polyuria is common. When, as commonly happens, the condition is complicated with catarrh of the ureter and bladder, a more or less abundant purulent sediment forms independently of the attack.

According to *J. Fischl*,[364] the urine at the commencement of this disease always contains casts, both hyaline and granular, and this authority regards their presence as of great importance in the differential diagnosis of pyelitis and cystitis. In addition, there occur plugs of cylindrical form composed of conglomerated white blood-corpuscles. These are probably derived from the renal pelves, and indicate an extension of the processes to the kidney proper, or pyelonephritis. When the latter condition is established by the appearances of pyelitis, then the presence of nephritis, and granular casts, renal epithelium, &c., may be observed.

3. Cystitis.—In uncomplicated cystitis the urine is generally pale, of normal sp. gr., and has an acid reaction, unless when alkaline fermentation takes place within the bladder. In the latter case it is turbid, and deposits on standing a more or less abundant deposit of fat-laden and swollen leucocytes and triple-phosphate crystals. Microscopically, moreover, pus cells and epithelium of various forms are to be seen. Amongst the latter, certain cells from the deeper layers of the mucous lining, provided with one or two finger-shaped processes, are especially noticeable (see p. 177). In connection with ichorous or hæmorrhagic

cystitis red blood-corpuscles and pigment masses appear in the urine. The complication of cystitis with an affection of the ureter cannot be determined by chemical and microscopical investigation, but its presence must be diagnosed from the existence of the other clinical symptoms.

Similar indications may be produced by a purulent urethritis, and confusion in this respect is to be guarded against.

The condition which has been designated as ammoniæmia, and which depends upon the absorption of ptomaines from the bladder, is often, but not always, accompanied with cystitis. In this connection the urine, when freshly voided, is undergoing alkaline fermentation.

4. Tuberculosis of the Urinary Organs.

(*a.*) **Tubercular Ulceration.** — Chemically and microscopically the urine in this condition resembles that of cystitis or pyelitis. It is pale, of normal sp. gr. and quantity, contains a variable proportion of albumin, and an abundant sediment, which consists principally of swollen and fat-laden pus-cells. The diagnosis must rest chiefly upon the recognition of tubercle-bacillus by the methods described in connection with the examination of the sputum (p. 75). In chronic tubercular affections the micro-organism is to be seen in great abundance in the urine, as in the specimen represented in fig. 89, and, as in this case, it tends to cohere in groups shaped like the letter S (comp. p. 191). The precise localisation of the affection must rest upon other grounds.

(*b.*) **Miliary Tuberculosis.**—In this affection the urine is often unchanged. It is apt, however, to contain blood at intervals, and may be distinguished from nephritis by the absence of casts and renal epithelium. Tubercle bacilli are *never* to be found *in considerable quantity* in the sediment.[365]

5. Calculus and Tumours of the Bladder.—These are to be suspected when copious intermittent hæmorrhages take place, and when the blood which is passed with the urine separates from it and is deposited in a thick layer at the bottom of the receiving vessel. The subjective symptoms, pain, &c., are usually sufficiently distinctive (see p. 205).

6. Catarrhal Urethritis.—The urine in catarrhal urethritis is altogether unchanged, except that the first flow contains pus. The affection is rarely met with. *Bockhart*[366] ascribes it to infection with non-specific vaginal secretion.

7. Gonorrhæal Urethritis.—The appearances are the same as in simple urethritis, but pus is usually very copious. It would appear that in all cases, while the infection is recent, specific micro-organisms, gonococci, are to be found. These were discovered by Neisser. They

are diminutive roll-shaped cocci, aggregated in large groups, which often closely pack the exfoliated epithelium cells of the urethra and cover their surface (*Neisser, Bumm, Bockhart*).[367] More recent experience has tended to lessen the clinical significance of these forms (*v. Zeissl, Hartdegen, Wendt*).[368] Bodies altogether resembling gonococci have been found to be present in the genital tract under the most dissimilar conditions. According to *Roux*,[369] however, the supposed gonorrhæal microbe may be distinguished from other forms by the fact that it does not stain with *Gram's* method. The specific character of gonococci admits of no doubt. Fig. 115 represents gonorrhæal pus from a case of old infection; fig. 116 shows gonococci from a preparation of *Dr. Kolisko*, made with the pus two days after the infecting coitus.[370] The occurrence of gonococci threads, and hyaline epithelium occurring in the urinary sediment of this condition is worthy of notice.[371]

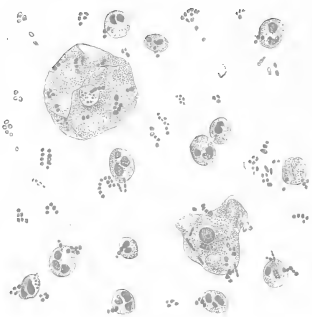


FIG. 115.—Urethritic Gonococci.

IV. The Urine in Diseases of the Alimentary Canal.—Diseases of the alimentary canal, for the most part, do not specially affect the urine pathologically; but in general it may be stated, that where they are attended with increased albuminous decomposition within the intestine, that fluid is apt to contain a large amount of indican. Carcinoma of the stomach with ulceration is occasionally attested by the appearance of peptone in considerable quantities (*Maixner*). In chronic catarrh of the stomach and especially in dyspepsia the acidity of the urine is apt to be much lessened.

V. The Urine in Hepatic Affections.—It may be stated in general that in all diseases which seriously involve the proper structure of the liver, the excretion of urea is diminished, and even in certain severe forms (acute yellow atrophy) entirely suppressed

(*Schultzen and Riess*).[372] It is then to some extent represented by other nitrogenous metabolites which appear in the urine, as leucin and tyrosin [373] (comp. p. 199), and together with these certain non-nitrogenous substances. Oxyamygdalic acid (*Shultzen and Riess* [374]), lactic acid, and the volatile fatty acids are often eliminated, as in cancer and syphilis affecting the liver (*v. Jaksch*).[375]

Diseases which cause an obstruction to the flow of bile are attended with the presence of bile-pigments in the urine (see p. 237).

In atrophic cirrhosis the latter is scanty, rich in urates, and almost or entirely devoid of biliary colouring-matters, but often contains a high percentage of urobilin. In hypertrophic cirrhosis it is often quite normal in quantity, sometimes increased, and exhibits abundance of bile-pigment.

The appearance of sugar and albumin in connection with disease of

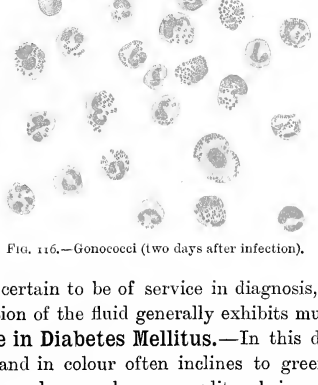


Fig. 116.—Gonococci (two days after infection).

the liver is too uncertain to be of service in diagnosis, and indeed the chemical constitution of the fluid generally exhibits much variety.

VI. The Urine in Diabetes Mellitus.—In this disease the urine is pale and clear, and in colour often inclines to green. Its quantity is enormously increased, as much as 12–15 litres being passed in twenty-four hours, sp. gr. high, ranging from 1.030–1.050. It is usually rich in the indigo-forming substance, and invariably contains a greater or less proportion of grape-sugar (p. 224). Towards the close of the disease albumin in considerable quantity is apt to make its appearance (*Stokvis*).[376] Exceptional cases of diabetes are met with in which the quantity of urine is not increased, and its sp. gr. is even lowered.

The author has met one such, in the practice of Professor Nothnagel, in which the urine contained much acetone, and showed a sp. gr. of 1.003. It was also found to hold more than 0.3 per cent. sugar in solution.

Other substances that are occasionally to be found in the urine of diabetes are acetone in large quantity, diacetic acid, and a number of other organic acids, such as β-oxy-butyric acid,[377] fatty acids,[378] &c.

In view of the fact that β-oxy-butyric acid occurs in the urine of diabetes, and of other conditions, as febrile states, some notice of the method of detecting that body is called for. The following is the best.[379] The grape-sugar in the urine is fermented with yeast, the fluid filtered, and the filtrate concentrated to a syrupy consistence. The latter is mixed with its own bulk of concentrated sulphuric acid, the mixture distilled, and the distillate collected in a test-glass. If β-oxy-butyric acid be present, crystals of α-crotonic acid separate on cooling from the contents of the test-glass, and may be recognised by determination of their melting-point (72° C.). Should no such crystals form in the process, the distillate is shaken up with æther, and the crystals which deposit on evaporation of the æther are tested to ascertain their melting-point.

β-oxy-butyric acid has also been found by *L. Hougounenq*[380] in the *post-mortem* blood of diabetics.

From recent observations of *Leo*,[381] it seems that such urine contains also a left-rotatory carbo-hydrate.

VII. The Urine in Diabetes Insipidus.—Marked polyuria is the characteristic symptom. Sixteen to twenty litres may be passed in the day. The urine is clear and almost colourless, of greatly reduced sp. gr. (1.0001–1.004). It contains neither albumin nor sugar, although it sometimes yields a small proportion of indican and inosite.

VIII. The Urine in Anæmia.—The fluid is pale, of low sp. gr., and neutral or alkaline in reaction. In the later stages of a severe anæmia albumin may be found, whilst at the same time, except for a few hyaline casts, tissue-elements are entirely absent from the sediment (Bamberger's hæmatogenic albuminuria).

In leukæmia the elimination of uric acid is increased.[382] *Prus*[383] has observed leucin, and lactic acid is also found in the urine.[384]

It will be convenient here to describe the method by which the latter body may be detected.

Detection of Lactic Acid.—The urine is evaporated on the water-bath to the consistence of a syrup, and extracted with alcohol. After the alcohol is distilled off or evaporated, the residue is extracted with æther, the æther distilled off, and the residue dissolved in water. To the solution a little basic acetate of lead is added. It is then filtered, the filtrate treated with sulphuretted hydrogen, again filtered, and finally digested on the water-bath. Lactic acid remains behind in the form of a syrup. From this the zinc salt may be obtained by the addi-

tion of carbonate of zinc. It may be recognised by its microscopical appearance (small prisms), or chemically by the proportion of water and of zinc respectively which result from its decomposition.

Colasanti and *Moscatelli*[385] assert that sarcolactic acid appears in the urine normally after great bodily exertion.

IX. The Urine of Toxic States.

1. Poisoning with Acids.—Poisoning with strong mineral acids—nitric, sulphuric, and hydrochloric—is generally followed by the appearance of blood and albumin in the urine. The symptoms, however, are often very transient. Toxic nephritis may ensue, and this especially when sulphuric acid was the poison. The urine is then scanty, of high sp. gr. and acid reaction. Chemically and microscopically, it presents the characters of that of acute nephritis (see p. 274). In every case of poisoning with acids which the author has had an opportunity of observing, the urine possessed the property of dissolving cupric sulphate in alkaline solution, and reducing it when subsequently boiled, whilst at the same time the most reliable tests besides gave no indication of sugar.

2. Poisoning with Alkalies.—After poisoning with caustic potash, which may be taken as the type of this condition, the urine passed during the first few hours contains albumin, invariably in severe cases, but very often also when the symptoms are slight. Its chemical and microscopical characters at the same time are not otherwise those of nephritis. Its reaction is feebly acid, seldom neutral, very rarely alkaline. It possesses the reducing property in a marked degree, while no evidence of sugar can be obtained with the phenylhydrazin reaction and other tests. Where the toxic agent has been chlorate of potash, acute nephritis is apt to follow. The detection of this salt in the urine may be effected by the method described at p. 113.

3. Poisoning with Metals and Metalloids.

(*a.*) **Lead Salts.**—In acute lead-poisoning, especially when attended with colic, large quantities of albumin are often transitorily present in the urine; more frequently still a true renal albuminuria, due to secondary nephritis, occurs. The presence of lead may be determined directly by the method described in connection with the vomit (see p. 113).

(*b.*) **Salts of Mercury.**—The urine in mercurial poisoning, after the lapse of a few hours, contains a large proportion of albumin, and very often blood. The symptoms of nephritis are usually sooner or later developed. This is especially true of poisoning with corrosive sublimate.[386]

Mercury may be detected in the same manner as in the vomit (p. 114), or in the urine *Fürbringer's* process may be employed,[387] or, better still, *Ludwig's* method.[388]

Five hundred cc. of urine are acidified with hydrochloric acid (1–2 cc.) in a beaker, and heated to 50–60° C. Three grms. of granular zinc or finely-divided copper are then placed in the fluid, which is shaken up for half a minute. The metal is then allowed to settle, the supernatant fluid poured off, and the sediment obtained upon a filter, where it is well washed with boiling water, and dried at 60° C. The powdered metal is then placed in a tube of hard glass, closed at one end, of 8–10 mm. diameter, covered with a plug of asbestos, upon which again is put a layer some 5–6 c. deep of granular oxide of copper; then another asbestos plug, and, finally, another layer of granular zinc, which has been previously dried and well heated. When the tube is filled in this way, it is drawn out to capillary calibre at a point some millimetres behind the last asbestos plug, and a bulbous expansion is made at the end. The cupric oxide is first heated to a dull red, the layer of zinc to a lesser degree, and, finally, the powdered metal containing mercury.

The mercury is deposited as a metallic powder in the capillary tube. The latter is then broken off above the last of the asbestos rings by letting water drop on it; a few particles of metallic iodine are placed in the first part of the tube while still hot, and the other expanded end of the capillary is connected with an aspirator (Böhm's air-pump serves best). The iodine vapour impinges on the mercury, and mercuric iodide forms, and is easily distinguished by its colour.[389]

Wolf and *Nega*[390] have employed a modification of this process. Organic matter is first removed by hydrochloric acid and chlorate of potash, and copper as a thin foil is introduced to receive the metal (mercury) instead of granular zinc or powdered copper. The method is said to be very reliable. That of *Alt*[391] is still more simple.

Almén's[392] method is the following:—About 300 cc. of the urine to be tested are taken, a little caustic soda and some sugar added, and the mixture boiled. The phosphatic sediment which falls carries the mercury with it. When it has entirely settled, the fluid is decanted off, the sediment dissolved in hydrochloric acid, and a piece of fine copper or brass wire placed in the fluid, which is then maintained at a moderate heat for an hour and a half. After this the wire is removed, boiled in alkaline water, and dried with blotting-paper. It is then placed in a glass tube of small calibre, which is broken a few millimetres in front of the wire, fused at the end, and heated over a small flame. The mercury sublimes, and is deposited in small globules, which can be readily recognised with the microscope. The reagents employed in the process should be previously tested for such mercury as they may themselves contain (see p. 114).

(*c.*) **Salts of Copper.**—In poisoning with copper salts, the urine is always reduced in quantity. It usually contains albumin, and very often also blood. As to whether acute nephritis can originate in this way some doubt exists. Experiments on animals would seem to favour the assumption. To detect the poison in the urine the method described at p. 115 will serve.

(*d.*) **Arsenic.**—In acute arsenical poisoning albumin and sometimes blood in considerable quantities appear in the urine. In one case the author has observed all the signs of acute nephritis. The urine pos-

sesses the reducing property, but sugar cannot otherwise be made evident. The effect on the urine of chronic arsenic-poisoning is but little understood. Albuminuria would seem occasionally to occur in this connection.

For the detection of arsenic, see chapter on the vomit (p. 115).

(*e.*) **Phosphorus.**—At the outset of a case of phosphorus-poisoning the urine is not notably changed as to quantity or sp. gr. Later it contains albumin, but not usually in considerable quantity, occasionally blood, and very commonly casts of different kinds. The presence of peptone has repeatedly been recorded (*Gerhardt, Maixner, v. Jaksch*). Biliary acids and colouring-matters are also met with, and Schultzen and Riess have detected sarcolactic acid. In a case which recently came under his notice, the author obtained an appreciable quantity of fatty acids from 300 cc. of the fluid. Tyrosin and leucin are rarely present, as is the case in acute yellow atrophy of the liver. Fat has been found in large quantities in the urine of phosphorus-poisoning.[393] The elimination of urea has been reported as exceeding or falling short of the healthy standard in different cases.

4. Poisoning with Alkaloids.

(*a.*) **Morphia.**—In acute poisoning with morphia the urine is commonly found to contain sugar. In chronic morphinism, again, it has a powerful reducing action, and sugar may sometimes be found with other tests. For the detection of morphia in the urine the method described at p. 117 may be adopted. It must be borne in mind, however, that morphia does not appear in the urine in all cases of morphia-poisoning or morphinism, and its absence does not prove that the alkaloid has not been taken. The researches of *Donath*[394] have shown that it may disappear entirely within the system.

(*b.*) **Nicotin.**—The toxic effects of nicotin are not associated with any special changes in the character of the urine. For the detection of the alkaloid see p. 118.

(*c.*) **Atropin.**—Little that is definite is known of this condition.

Atropin may be isolated from the urine in the same manner as that described in connection with the vomit (p. 118). As a test for its presence a little of the urine may be placed in an animal's eye, and the mydriatic effect watched for. This will be obtained, according to *de Ruitter* and *Donders*,[395] when the urine contains the alkaloid in the proportion of one part to 130,000 of water.

(*d.*) **Ptomaines (Exogenic Toxicosis).**—The phenomena of poisoning with ptomaines need further investigation. In a case (of sausage-poisoning) which was recently under the care of Professor Nothnagel, albuminuria and the signs of nephritis supervened.

5. Poisoning with Ethylic Alcohol.— Chronic alcohol-poisoning

appears to produce nephritis and arterio-sclerosis. In the acute condition the poison can be found only as the merest trace in the urine.[396] To recognise its presence the urine must be distilled by means of a steam-bath, and the distillate treated in the manner described at p. 121.

6. Chloroform-Poisoning.—In this condition the urine is generally of high sp. gr. It often contains a trace of albumin and some sugar. For the detection of chloroform the urine is distilled by means of a steam-bath to prevent frothing, and the first drops of the distillate are submitted to Hoffmann's or Vitali's test (see p. 121). The results obtained in this way are equally useful with those of *Maréchal's* method.[397]

7. Carbolic Acid-Poisoning.—When large quantities of carbolic acid have been administered through the mouth or absorbed from a wound, the voided urine assumes a dark-green colour, which changes to black on standing. This colour is due to the presence of hydrochinon, a derivative of phenol, which, while still within the system, is in part transformed into coloured oxidation products (*Baumann* and *Preusse*).[398] Pure phenol is never present, but even in the severest cases is found only in combination with sulphuric acid (p. 244). Hence the characteristic reaction of carbolic acid—a violet coloration with solution of perchloride of iron—cannot be obtained with the urine. The latter contains a small quantity of albumin, and commonly also hæmoglobin (p. 223). A further evidence of the condition depends upon the diminished proportion of simple sulphuric acid which it contains. When chloride of barium is added to healthy urine acidulated with acetic acid, an abundant precipitate of barium sulphate falls; but when from any cause the quantity of sulphuric acid in the uncombined form is lessened, this precipitate fails, or is represented by a mere turbidity. If now in this case the urine be filtered and boiled with hydrochloric acid, so as to decompose the phenol-sulphuric acid (see p. 244), with the reproduction of simple sulphuric acid a copious precipitate of barium sulphate forms.

Inasmuch as phenol-sulphuric acid is normally a constituent of the urine, it serves but little purpose in cases of carbolic acid-poisoning to attempt an estimate of the phenol which passes over as tribromo-phenol (see p. 246) in the process of distillation. A better plan is to determine the relative proportion of simple and compound sulphuric acid present (see p. 246). If it be found that the latter is increased while the first is diminished, and if at the same time such affections as promote the elimination of æther-sulphuric acids (active albuminous decomposition) can be excluded, this is strong presumptive evidence of carbolic acid-poisoning.

The same method will serve for the detection of all the aromatic substances which appear in the urine as æther-sulphuric acids in cases of poisoning or after the administration of drugs.

8. Poisoning with Nitro-Benzol and Aniline.

(*a.*) **Nitro-Benzol.**—In cases of poisoning with this substance, the urine has the odour of nitro-benzol, and generally contains a substance which has the property of rotating polarised light to the left, and of reducing cupric sulphate in alkaline solution.[399]

(*b.*) **Aniline.**—The recorded observations of cases of poisoning with aniline show that the character of the urine varies considerably. It is usually dark in colour and highly concentrated (*Grandhomme*).[400] In one instance (*Fr. Müller*[401]) it was free from sugar, albumin, and blood, and exhibited the reduction phenomenon in a marked degree. Æther-sulphuric acids were notably increased. An æthereal extract was found to contain aniline by its yielding a violet colour in presence of solution of chloride of lime (see p. 123). *Müller* is of opinion that this substance is in part eliminated as paraamido-phenol-sulphuric acid.

9. Poisoning with Carbonic Oxide Gas.—The urine passed in this condition invariably contains grape-sugar[402] and an uncertain proportion of albumin. The quantity of sugar varies directly with the intensity of the poisoning.

V. THE DETECTION OF CERTAIN DRUGS IN THE URINE.

1. Iodoform and Salts of Iodine and Bromine.—After the exhibition of iodoform, whether internally or by outward application, iodides and iodates may be detected in the urine. So too with iodine when applied externally as the tincture, or taken by the mouth as iodide of potassium, it may be readily recognised in that fluid.

To *test for iodine:* the urine is treated with a little fuming nitric acid or chlorine water, and shaken up with chloroform. If a salt of iodine be present, the metal is set free, and dissolves in the chloroform, with the formation of a red colour.

The *quantitative estimation* may be effected best by *Harnack's*[403] method, in which all the iodine is converted into palladium iodide. Iodine appears in the urine very shortly after it has been taken into the system. A quarter of an hour will suffice for its manifestation there.

Bromine salts, when very abundant, may be detected thus:—The urine is treated with chlorine water, and shaken up with chloroform, when bromine dissolves in the latter, with the production of a yellow colour. It will usually be found necessary, however, to evaporate the urine previously, then to incinerate it carefully, and to test the colourless watery extract from the ash in the manner described above.[404]

2. Salicylates.—Salicylates also very quickly pass into the urine. The latter then exhibits a remarkable reducing power, and yields a violet colour in presence of solution of perchloride of iron. This reaction depends partly upon the presence of salicylic acid and partly upon that of salicyluric acid, into which the latter is changed within the system. This reaction is not readily prevented by boiling. The body on which it depends is taken up by æther from acidified urine, and can be detected in the æthereal extract by means of solution of perchloride of iron. The reaction, unlike that of diacetic acid (see Diaceturia), does not disappear on standing. It is well in testing for it to precipitate the phosphates with solution of perchloride of iron and filter, and then test the filtrate with more of the reagent.

The exhibition of salol (phenyl-æther of salicylic acid) imparts a similar character to the urine, which also on standing acquires a tint varying from dark-green to black, like that following the use of carbolic acid.

The use of betol (naphthalol, salicylate of β-naphthol-æther) does not impart any special colour to the urine, which, however, yields the perchloride of iron reaction. Both salol and betol appear in the urine as sulphuric acid combinations (*v. Jaksch*).

3. Quinine, Kairin, Antipyrin, Thallin, Antifebrin, and Phenacetin.

(*a.*) **Quinine.**—This alkaloid is said by *Kerner*[405] to be eliminated as dioxyquinin. It renders the urine dark. For its detection a large quantity of the fluid is taken, treated with ammonia, and shaken up with æther. The latter is then distilled off or evaporated, and quinine remains in the residue. It is dissolved in acidulated water, and the addition of chlorine water and ammonia causes an emerald-green tint to develop.

(*b.*) **Kairin.**—Under the influence of this drug the urine acquires a brown colour, which becomes brownish-red with solution of perchloride of iron. The substance upon which this reaction depends may be extracted with æther from acidified urine. The reaction in the æthereal extract remains even after the lapse of weeks. The addition of strong acids to the urine destroys the reaction, and it is, moreover, impaired by prolonged boiling. According to *v. Mering*,[406] kairin is eliminated as kairin-potassium sulphate.

(*c.*) **Antipyrin** imparts a darker tint to the urine, which gradually becomes a purple-red when treated with perchloride of iron. If the urine be acidulated and extracted with æther, a substance is obtained which colours brown with perchloride of iron. This reaction is gradually lost after the lapse of a few days. It is impaired, but not destroyed, by boiling the urine. It is destroyed by the addition of acids. Esti-

mation of the quantities of simple and of æther-sulphuric acids in appropriate instances has shown that antipyrin is eliminated as a sulphuric acid combination (*v. Jaksch*).

(*d.*) **Thallin.**—The urine after the administration of thallin is usually a brownish-green when viewed in bulk, greenish in a thin layer. Treated with perchloride of iron, it presently exhibits a purple-red tint, which, after the lapse of four or five hours, if undisturbed, passes into a brownish-red. If a little mineral acid be added and the fluid be shaken up with æther, the latter takes up a substance which colours brown-red with perchloride of iron. The coloration does not vanish when the specimen is allowed to stand, but rather becomes more marked. When the pure thallin urine is shaken up with æther, the æthereal extract contains a body which colours green with perchloride of iron (thallin).[407] The tint in this case disappears on prolonged standing. The red colour obtained with perchloride is lost by boiling for a few seconds. It is likewise destroyed by mineral acids. Thallin is partly eliminated in the form of chinanisol.

(*e.*) **Antifebrin.**—The physical character of the urine is unaffected even by large doses of antifebrin. Chemically it has been ascertained by F. Müller that it contains more than the normal proportion of æther-sulphuric acids; and this is accounted for by the supposition that antifebrin is changed within the system to paraamido-phenol-sulphuric acid. Where the drug has been exhibited in large quantities, it may be recognised in the urine (*F. Müller*[408]) thus :—The fluid is boiled with one-fourth its bulk of strong hydrochloric acid, and when it has cooled, a few cc. of a 3 per cent. solution of carbolic acid is added, and then a few drops of a solution of chromic acid. If paraamidophenol be present, the specimen develops a red colour, which gives place to blue on the addition of ammonia.

For the detection of antifebrin *Yvon*[409] recommends the following method :—The urine is shaken up with chloroform, and the residue from the extract is heated with a little mercurous nitrate. If antifebrin be present an intense green coloration takes place. When antifebrin has been separated from the urine, as by shaking it up with æther and acid, it can also be recognised by the addition of chloroform and caustic potash. Antifebrin also is excreted for the most part in combination with sulphuric acid (*Fr. Müller, Mörner, v. Jaksch*). According to *Mörner*,[410] this body is in part oxidised to acetylparaamidophenol within the system, and eliminated as æther-sulphuric acid.

(*f.*) **Phenacetin** (acetphenetidin).—The colour of the urine is unaffected by this body, even in large doses. It rotates polarised light to the left (glycuronic acid combination, *Fr. Müller*[411]), and exhibits the paraamidophenol reaction described above. It contains no free

phenacetin, but the presence of phenetidine may be shown by changing it into its diazo compound, which then yields a purple coloration with naphthol or yellow with phenol (*Müller*). The process is as follows:—To a specimen of the urine two drops each of hydrochloric acid and of sodium nitrite solution (1 per cent.) are added. The further addition of an alkaline watery solution of α-naphthol and a little caustic soda causes a beautiful red colour to develop, and this passes into violet if hydrochloric acid be supplied. Under the same conditions phenol gives a citron-yellow in alkaline and a rose colour in acid solution. When large doses of the drug have been taken, the urine gradually takes a brown-red tint in presence of perchloride of iron solutions and oxidising substances, changing slowly to black on standing for a long time.

4. Chrysophanic Acid.—The administration of infusion of senna or of preparations of rhubarb imparts a reddish-brown colour to the urine, which may be present in the freshly-voided fluid or develop on standing. This gives place to red on the addition of alkalies at ordinary temperatures. When boiled with alkalies the resulting phosphatic sediment is not red, but yellow; and if dissolved in acetic acid the solution also colours yellow, changing to violet on exposure to the air; in this respect differing from the precipitate of blood-pigments, which is also soluble in acetic acid, but gradually bleaches when exposed.

5. Santonin.—Santonin taken internally colours the urine yellow, which again gives place to red on the addition of alkalies. Its presence may be discriminated from that of rhubarb (*Munk*[412]) from the fact that the red colour developed by the latter in presence of alkalies, though permanent, is rapidly destroyed by reducing agents (granular zinc, sodium amalgam), whilst that due to santonin persists under like circumstances. Chrysophanic acid is precipitated with baryta water. The precipitate is red and leaves a colourless filtrate. The latter is yellow if santonin be present. The addition of alkaline carbonates turns the urine red, rapidly if it contain rhubarb, very slowly and gradually in the case of santonin.

G. Hoppe-Seyler[413] has suggested the following method for the discrimination of chrysophanic acid and santonin:—The urine is treated with caustic soda and extracted with amylic alcohol. If the colouring matter of santonin be present, it passes over with the alcohol, and the specimen is decolorised. The colouring matter of chrysophanic acid derived from the exhibition of rhubarb or of senna is little or not at all taken up by amylic alcohol in presence of the alkali.

6. Tannin.—When tannin has been taken medicinally in large doses, the urine turns dark-green with solution of perchloride of iron.

7. Naphthalin.—Naphthalin in large doses causes the urine to

assume, especially on standing for a long time, a dark tint, like that due to carbolic acid.

According to *Penzoldt*,[414] a dark-green colour develops rapidly in presence of concentrated sulphuric acid.

8. Copaiba Balsam.—Copaiba balsam in the urine yields a red colour with hydrochloric acid, changing to violet when heated. If ammonia or caustic soda be added to the urine, a light-brown colour with a blue fluorescence develops (*Edlefsen*).[415]

When boiled with an acid the urine gives a precipitate which is soluble in alcohol.

It may be mentioned here that after the use of oil of turpentine the urine sometimes gives a precipitate with acids. It has also a characteristic odour of violets.

CHAPTER VIII.

INVESTIGATION OF EXUDATIONS, TRANSUDATIONS, AND CYSTIC FLUIDS.

Fluid may be effused into any of the cavities of the body as a consequence of inflammation or of disturbances in the circulatory system.

Under appropriate circumstances a portion of such fluid may be drawn off by puncture or in some other way, or a spontaneous opening may occur, and in either case the fluids so obtained may be submitted to examination. In this way much useful information may be secured for the purposes of diagnosis. A question arises at the outset as to whether the fluid is an inflammatory product (exudation), or the result of impediment to the circulation, or derived from the degeneration of certain organs (transudation).

A.—EXUDATIONS.

An exudation may be purulent, sero-purulent, putrid, hæmorrhagic, or serous. All such fluids, with the exception of the last two, imply, of course, an inflammatory origin. Upon its other characters, and especially upon the nature of the tissue elements which it contains, more precise inferences may be based in the case of any one of them.

1. Purulent Exudations.

I. NAKED-EYE APPEARANCES.—Pus (*bonum et laudabile*) is a turbid fluid of varying consistence and high sp. gr., with an alkaline reaction, and ranging in colour from grey to a greenish-yellow. It may accumulate in natural cavities (exudations), or be effused amongst the tissues (phlegmon), or, finally, it may be secreted from the surface of a wound. On standing in a cool place it separates into two layers, the upper of which is of a light-yellow colour and tolerably transparent, and the lower opaque from the deposit of pus-cells. It is often brown or brownish-red from admixture with blood. Putrid pus can always be discriminated by its naked-eye properties. It is thin, green or brownish-red, and emits an extremely penetrating odour of indol and skatol.

II. MICROSCOPICAL CHARACTERS.

1. White and Red Blood-Corpuscles and Epithelium.—When pus is submitted to microscopical examination, it discloses a multitude of cells which entirely resemble white blood-corpuscles. When derived from a perfectly fresh specimen, these cells exhibit the contractile property, and the mahogany coloration obtained with solution of iodine and iodide of potassium or ammonium attests the presence of glycogen. This reaction is obtained best with fresh pus from the surface of a wound. The dead cells are shrunken and very granular, or may appear as decomposed or decomposing particles of protoplasm.

In addition to this, giant pus-corpuscles and fat-laden cells are occasionally observed. No special significance attaches to their presence. They have been seen in the pus from an abscess of the gum (*Boettcher*[1]), in hypopyon (*Bizzozero*[2]), and in the contents of suppurating ovarian cysts (*v. Jaksch*).

Some red blood-corpuscles are nearly always present in freshly-secreted pus, and when blood in considerable quantity has been effused and afterwards its elements disintegrated, the discharge may be more or less amply tinged by admixture with blood-pigment or hæmatoidin crystals.

Fatty particles and globules are seldom wanting, either free or combined with the protoplasm of the cells. The epithelium elements are comparatively few. In cancerous exudation from the pleural cavity vacuolated epithelial and fatty endothelial cells are commonly to be met with.

2. Fungi.—Modern research[3] has established the fact that the formation of pus in animal organisms is effected almost entirely through the agency of micro-organisms, and that such bodies can nearly always be detected in the discharge with the aid of the staining methods (p. 315) which the recent advances of science have placed within our reach, where the microscope alone fails to disclose their presence.

Important experiments upon animals[4] have shown also that suppuration may be caused by certain chemical substances, as cadaverin, croton oil, &c., independently of micro-organisms; and it is possible that the human system may be similarly affected by them.

1. Micrococci.—Micrococci of varying form and size are very often to be seen in fresh pus.[5] Fig. 117, which represents the appearance of a specimen taken from a pleural exudation and stained by Gram's method, affords an appropriate illustration. They are usually arranged in chains (Streptococci), occasionally in pairs (Diplococci).

Passet,[6] proceeding on Koch's method, has cultivated no less than eight different forms of fungi from pus. When suppuration has continued for a long time in a cavity excluded from the air, micro-organisms

are sometimes wanting in the pus. *Brieger*[7] has observed Staphylococcus pyogenes aureus and Streptococcus pyogenes in pus from a woman with puerperal fever. The micro-organisms hitherto mentioned are of little importance, but the detection of certain pathogenic fungi in the pus is of the utmost consequence. *Bujwid*[8] lately made the interesting discovery that the effect of grape-sugar upon the tissues is to promote the development of Staphylococcus aureus by diminishing their resistance, and so to favour the formation of pus. The tendency of diabetics to undergo suppurative processes, so long a matter of clinical observation, is explained in this way.

A blue colour has occasionally been observed on the surface of suppurating wounds. This is produced by colonies of Bacillus pyocyanogenus or of a fungus resembling it. The colouring-matter has been isolated from such pus in combination with hydrochloric acid.[9]

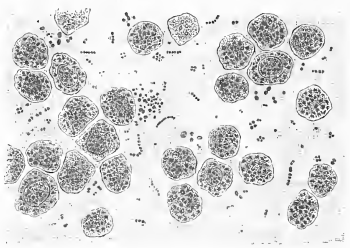


FIG. 117.—Micro-organisms of Pus.

2. Tubercle Bacillus.—The tubercle bacillus has often been discovered in tubercular pus,[10] but the author has sometimes failed to find it even in the fresh discharge. Its presence, of course, is conclusive as to the nature of the disease, but its absence does not imply that no tubercle is present. It would appear that under certain conditions the bacillus rapidly disappears from fresh discharges (*Metschnikoff*).[11]

3. Bacillus of Syphilis.—The bacillus discovered by *Lustgarten*[12] in the pus of syphilis affords a valuable indication of this disease; but caution must be observed in identifying it, since *Alvarez* and *Tavel*[13] have shown that certain secretions, as the smegma præputiale and vulvare, are apt to contain forms which closely resemble the venereal microbe. Such forms are to be discriminated by the behaviour of stained preparations in presence of alcohol. In the case of the bacillus of syphilis, these are with difficulty and very slowly bleached by alcohol, whilst the microbe of smegma readily loses its colour under the action of that substance.

An important addition to our knowledge of the bacillus of syphilis was recently made by *Kamen*,[14] who found the micro-organism in the sputum of a child of nine years. The character of *Lustgarten's* bacillus has been much questioned of late, and others have regarded certain cocci as the specific excitants of syphilis (*Kassowitz*, *Hochringer*, *Disse*, *Taguchi*).

For the detection of the bacillus of syphilis *Lustgarten*[12] proceeds as follows:—The cover-glass preparation is immersed in an Ehrlich-Weigert gentian-violet fluid for 12–24 hours at the ordinary temperature. It is then removed, rinsed for some minutes with absolute alcohol, and placed for ten seconds in a $1\frac{1}{2}$ per cent. solution of permanganate of potash, after which it is treated with a watery solution of pure sulphurous acid, and finally washed with water. Should it happen after this that the preparation still shows colour, it is again placed in

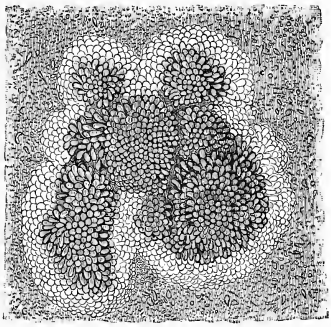


Fig. 118.—Actinomyces Granules (slightly magnified).

permanganate of potash for three or four seconds, and afterwards in sulphurous acid until all colour has disappeared, the remainder of the process also being repeated. It is to be noted that other microbes, both pathogenic and innocuous, are stained by Lustgarten's process as well as that of syphilis.

De Giacomi[15] has suggested a method which is very serviceable for the detection of the bacillus of syphilis. In this the dried cover-glass preparation is warmed for some minutes in an aniline-water fuchsin fluid, then washed with water to which a few drops of perchloride of iron solution have been added, and finally decolorised in a concentrated solution of that salt. The bacillus then remains of a red colour, while all other micro-organisms are bleached.

4. **Actinomyces.**—This parasite was first discovered by *Bollinger*[16] in

cattle, and afterwards in man by *Ponfick*[17] and *Israel*.[18] In the former it gives rise to tumours of considerable size, but in man its proliferation is usually associated with chronic inflammation and the production of pus.

It would appear from numerous communications that have been made in recent years that actinomycosis is a disease of very wide distribution,

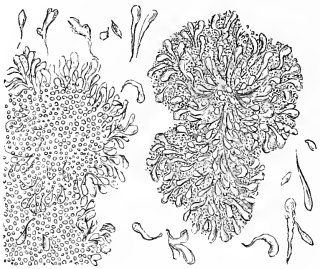


Fig. 119.—Actinomyces Granules (compressed).

and that amongst its symptoms is a severe form of angina, till lately obscure, to which the name of angina Ludovici has been given.[19]

The pus of this disease is thin, viscous, and somewhat tenacious, and discloses to the naked eye small nodules of a grey or yellow colour, and about the size of a poppy-seed. Under a low power of the microscope these particles appear as a dense bunch-like aggregation of little

Fig. 120.—Actinomyces (unstained preparation).

balls, which under a higher magnifying power are seen to consist of pear-shaped, and radially arranged masses, which have a considerable refractive power (figs. 119, 120). Towards the centre of such masses the individual elements diminish in size and fade into a fine network of ramifying fibres. If one of the nodules be bruised, it shows in the first place numerous detached club-shaped forms, having a radiat-

ing disposition toward the periphery (fig. 119), and passing gradually at the centre into a sort of detritus; and in addition to these are a variety of other objects, club-shaped and of indeterminate appearance (degeneration types of the fungus), lying apart.

There was for a long time much doubt as to the botanical character of Actinomyces. Recently, however, it has been established [20] that it belongs to the class of fission-fungi, being a fission-alga (Cladothrix). The characteristic club-shaped bodies must be regarded as degeneration forms of the fungus. In unstained microscopical preparations they may be seen sometimes to fade into the central fine network, and even occasionally to be enclosed in its meshes (fig. 120).

With the aid of staining methods (*Gram's* is the best) the individual threads of the network are readily distinguishable. They present a jagged or undulating contour, and result from the cohesion of a series of minute spherical organisms, connected together by a remarkably delicate envelope. The centre, to which all the constituent threads of

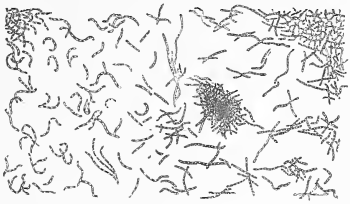


FIG. 121.—Actinomyces (stained by Gram's method).

a group converge, is occupied only by a very dense network of this formation (fig. 121).

The pear-shaped bodies above alluded to may be more clearly defined by staining with *Weigert's* [21] process. For this purpose a solution is made by adding together 20 cc. absolute alcohol, 5 cc. concentrated acetic acid, and 40 cc. of distilled water, and to the mixture so much of the so-called French extract of litmus as will give the fluid a dark-red colour, remaining ruby-red after repeated filtering (*Wedl's* [22] litmus solution). In this solution the cover-glass preparations are allowed to remain for an hour or so, then lightly rinsed with alcohol, and placed for two or three minutes in a 2 per cent. gentian-violet fluid, which should be boiled before use, and filtered after cooling.

If the specimen be now examined, it will be found that whilst the central mass of Actinomyces is colourless, the fungus threads are stained a ruby-red. *Baranski* [23] advocates staining with picro-carmine. In most cases, however, the character of this fungus can be determined

by a simple microscopical examination. The physical qualities of the pus, and the discovery in it of groups of Actinomyces or the club-shaped degeneration forms of the parasite, are points which will aid the diagnosis. In particular cases it may be necessary to resort to Gram's staining method, and to observe the minute structure of the ultimate threads of the fungus, as noticed above (comp. p. 295).

5. Bacillus of Glanders.—The specific micro-organism may be found in the pus from the ulcerated nasal passages in glanders. The method described in connection with the examination of the blood (p. 32) will serve for its recognition here.

Another method has recently been suggested (*Löffler*).[24] An aniline-water gentian-violet fluid or a concentrated alcoholic solution of methylene-blue may be employed, and immediately before use it is added to its own bulk of a (1 : 10,000) solution of potash. In the fluid which results the cover-glass preparation is immersed for five minutes. It is then removed and placed for a second in a 1 per cent. solution of acetic acid, tinged slightly yellow with oo-trapæolin. By means of another mixture, containing two drops of concentrated sulphurous acid and one of a 5 per cent. solution of oxalic acid in 10 cc. of water, the alkaline staining fluid may be decomposed and the preparation freed from colour. In this way the micro-organisms are very beautifully stained.

The bacillus of glanders may also infest the pus from an abscess. In any case, its character may usually be sufficiently determined in stained specimens by means of the microscope. When necessary, however, all doubt may be removed by observing the result of inoculating animals with the microbe. In the intermediate process of cultivation certain characteristic properties are disclosed. When the various other micro-organisms which are nearly always present in the pus have been eliminated by Koch's method (p. 321), the pure plate-cultivation crop made in nutrient agar-agar at 37° C. has the appearance of a greyish-white drop. The pure cultivation-product, when inoculated upon animals, as the mouse or guinea-pig, induces glanders. When engrafted on the potato and kept at a temperature of 35° C. the bacillus forms, in two or three days, a thin greasy coating of a brown colour. In coagulated blood-serum at a low temperature it develops after two or three days in the form of small scattered transparent drops, which have a colour very much the same as that of the serum. The fungus is readily cultivated in glycerine agar-agar[25] and in nutrient milk-peptone.[26] When allowed to rest for some time longer, the cultivations are said to develop spores, but this point is not established (*Baumgarten*).[27]

6. Bacillus of Anthrax.—Opportunity occasionally offers of examining the pus derived from a carbuncle in anthrax. The specific

micro-organism is that described at p. 29. A certain familiarity with its life-habits will often facilitate its recognition, especially where it can be obtained only in very small numbers. The process by which these have been studied is in general the same as that employed for other pathogenic microbes. Thus the elimination of other forms is effected by Koch's plate (nutrient agar-agar and nutrient gelatine) and inoculation methods (see p. 322). When cultivated in a nutrient gelatine medium, the bacillus of anthrax develops in twenty-four to thirty-six hours, and forms minute points which are scarcely visible to the naked eye. With a lens the colonies are seen to be dark figures with an irregular undulating outline. Their peculiar shape becomes more evident after the lapse of forty-eight hours, and later the cultivation liquefies, and from the dark central point it stretches in wavy strings over the entire surface of the plate.

The fungus does not liquefy agar-agar. When cultivated on sterilised potato, it forms a whitish-grey slimy patch of uneven surface, and extending but a few millimetres from the site of inoculation. Blood-serum cultivations form a superficial coating of a white colour. Nutrient gelatine test-tube cultivations take the form of delicate white threads much interwoven, and gradually liquefy the gelatine; drop-cultivations of anthrax bacillus in nutrient broth take the shape of long threads, in which, after a time, lustrous spots (spores) develop at regular intervals (comp. p. 29).

If the bacillus be imparted by inoculation to such animals as the mouse or guinea-pig, the animals exhibit the symptoms of splenic fever, and the micro-organism may be obtained from their blood.[28]

7. Bacillus of Leprosy.—The nodes which are apt to form on various parts of the skin and mucous membrane in leprosy occasionally break down and ulcerate, with the formation of an abundant thin pus. In this, as indeed in the growths of leprosy generally, large numbers of bacilli may be found (*A. Hansen, Neisser*).[29] They have the form of minute rods 4–6 μ long and 1 μ in breadth, and have a close resemblance to the bacillus of tubercle (see p. 77). Like the latter, they stain in alkaline fluids, and are not bleached by subsequent exposure to acids. They are distinguished by the comparative readiness with which they stain, and by more easily taking up the colouring-matter from a simple watery solution of aniline dye.[30] For their detection in the pus a suitable dry cover-glass preparation must be made (see p. 76), stained with Ziehl-Neelsen's carbol-fuchsin fluid, and again decolorised in acid (best with nitric acid) alcohol.

Melcher and *Ortmann*[31] have produced leprosy in animals (rabbits) by inoculation with diseased tissue; and *Bordoni-Uffreduzzi*[32] has cultivated the fungus from the spinal cord of lepers by inoculation in

glycerine peptone-serum, glycerine blood-serum, and glycerine agar-agar. When cultivated by surface inoculation of nutrient substances, it forms ribbon-like cultivations with irregular borders, and of a light-yellow colour. On glycerine agar-agar it develops in small round whitish-grey cultivations, somewhat elevated at the centre, thinned at the edges, and having a jagged outline.[33]

3. Infusoria.—Little is known as yet as to the presence of these parasites in pus. *Künstler* and *Pitres*[34] found numerous large spores with ten to twenty crescentic corpuscles in the pus from the pleural cavity of a man. These bodies closely resembled the coccidia which occur in the bodies of mice (comp. pp. 61, 141). *Litten*[35] observed cercomonads in the puncture-fluid, derived probably from the lungs.

4. Vermes.—Filaria have been observed occasionally as occurring in the pus of tropical abscess of the liver.[36] In temperate climates also abscesses result from an invasion of hydatids, and in such cases the pus contains entire echinococcus cysts, or fragments of the membrane and hooklets. *Babesiu*[37] found filaria in the gastro-splenic omentum. They were probably the same as *Grassi's*[38] Filaria inermis. With these should be mentioned the bodies resembling filaria which *Sarcani*[39] reports as found by him in the suppurating parotid of a woman.

5. Crystals.

1. **Cholesterin.**—Crystals of cholesterin are very rarely to be found in fresh pus—more commonly in that derived from cold abscesses, and most abundantly in fœtid discharges and in suppurating ovarian cysts. For their character and recognition the reader is referred to pp. 82, 204.

2. **Hæmatoidin.**—This body exhibits the same variety of form here as in the sputum, the urine, and the fæces (pp. 81, 155, 196). Its presence invariably points to a previous hæmorrhage. It is especially abundant in the case of suppurating hydatid cysts.

3. **Fatty Needles.**—These are of very many shapes and forms, occurring sometimes singly and sometimes in groups and clusters. They are the outcome of a degenerative process, and show that the pus in which they are found has been long formed. Very perfect margarin needles may be obtained from gangrenous pus (fig. 122).

4. **Triple Phosphate.**—Crystals of triple phosphate are a common constituent of the pus (see p. 197). Crystals of carbonate and of phosphate of lime are also met with, and most commonly in fœtid pus.

III. CHEMICAL EXAMINATION OF THE PUS.—It rarely happens that much aid accrues to diagnosis from the chemical examination of the purulent discharges. The albuminous substances which may commonly be recognised are serum-albumin, globulin, and especially

peptone (*Hofmeister*[40]) in large quantity. The latter is derived from the cells, not from serum. For the means of detecting these bodies the reader is referred to p. 211.

Fresh pus always contains glycogen, and traces of grape-sugar are seldom wanting. In testing for the latter, the pus should be boiled with an equal weight of sodium sulphate, to free it from albumin, filtered, and the filtrate treated in the manner previously described (p. 48).

In cases of jaundice the pus may contain bile-pigments and biliary acids.

Considerable quantities of nuclein, fats, cholesterin, and a number of inorganic salts, notably phosphate and chloride of sodium (*Miescher*, *Naunyn*[41]), are constantly to be found.

In three specimens of pleuritic exudation which he has investigated the author found abundance of acetone, and it would appear from a private communication of Professors Baumann and Baümler that that body is commonly a constituent of pus. In another specimen submitted to him by Dr. R. Paltauf, whose attention had been attracted by the evident odour of acetone, it was found by the author to be plentifully present; and the researches of others have shown that acetone is frequently present in large proportion in exudation fluid. Fœtid pleural exudation often contains sulphuretted hydrogen. The method for its detection is described at p. 272. There, as in certain forms of hydrothionuria, fungi possessing the property of liberating sulphuretted hydrogen may be isolated from the fluid.

Guttmann[42] found indigo-producing substances in exudations.

2. Sero-Purulent Exudations.

Sero-purulent closely resemble the purulent discharges in their chemical, physical, and morphological character. They are distinguished chiefly by the relatively smaller proportion of extractives which they yield. They invariably point to antecedent inflammation.

3. Putrid Exudations.

Discharges of this character are brown or brownish-green in colour, and have a penetrating and disagreeable odour. Their reaction is usually alkaline. Microscopically they exhibit much-shrunken leucocytes and an abundance of crystals, chiefly of fat, and of cholesterin and hæmatoidin in lesser proportion. They also contain a profusion of fission-fungi of different kinds (see fig. 117).

4. Hæmorrhagic Exudations.

A hæmorrhagic exudation contains an abundance of red blood-corpuscles, and often also a considerable quantity of dissolved hæmoglobin. Endothelial cells loaded with fat are almost invariably to be found, and when these occurring in pleuritic fluid exhibit the glycogen reaction in a marked degree, they favour the assumption of carcinoma (*Quincke*).[43] A positive diagnosis upon this point results from the further discovery of cancer-cells.

In the absence of determinate (specific) elements, as cancer-cells, tubercle bacillus, &c., no very definite conclusion can be drawn from the hæmorrhagic character of the discharge, since many different pro-

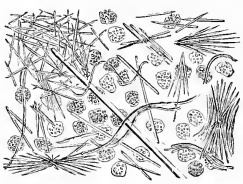


Fig. 122.—Putrid Exudation.

cesses may be associated with the effusion of blood. Still, in the case of pleuritic fluid, where scurvy and cancer of the pleura can be excluded, the appearances would point to tuberculosis.

5. Serous Exudations.

Fluids of this class are almost quite clear and more or less deeply tinged yellow; they coagulate on standing (for twenty-four hours), and yield a clot which is usually rich in fibrin. Microscopically they exhibit scattered red blood-corpuscles in usually fairly good preservation, but sometimes much attenuated, a number of leucocytes, some fatty globules and endothelial cells, separately or in groups. In addition to these are sometimes cells ranging from 7–30 μ in diameter, and formed of very small droplets. These may contain two or three large cavities (*Bizzozero*).[44]

There is reason to believe that micro-organisms very frequently infest the serous exudations, but the subject calls for further elucidation. It would appear, at any rate, that fungi occur oftener in them than in the transudations which so closely resemble them in their physical and chemical properties. The fluid obtained from the pleural cavity in tubercle of the pleura with breaking down of tissue may contain the specific bacillus of that disease. In cases, however, where

no discharge of tubercular matter has taken place into the pleural cavity the tubercle bacillus will not be found.

Cholesterin crystals occur in serous discharges of old standing. Chemically serous exudation fluid contains serum-albumin and globulin, but no peptone. Sugar in small quantity is invariably present,[45] and acetone from time to time.

The density of the fluid is clinically a fact of much consequence. It may be estimated by means of a picnometer, or with an accurate aërometer, regard being had at the same time to conditions of temperature. The sp. gr. will usually be found to exceed 1.018 (*Reuss*).[46]

6. Chylous Exudations.

Peritoneal exudation is commonly characterised by the abundance of fatty matter which it contains. This also is especially true of discharges depending upon obstruction of the thoracic duct. [See Appendix XXIII.]

The appearance of chyle, however, is sometimes misleading, since it has been shown that this character belongs in general to pathological fluids of low sp. gr., especially to such as owe their origin to passive congestion (*F. A. Hoffmann*).[47]

In general it is a difficult task to determine whether a particular fluid effused into one of the cavities of the body is due to inflammation (exudation) or to simple obstruction (transudation). In such cases a probable conclusion may be based upon the specific gravity of the fluid (*Méhu, A. Reuss*).[48] A further point of interest in this connection is the fact that inflammatory fluids are relatively rich in fibrin (for the estimation of this body see p. 45), and in dry residue products.

B. TRANSUDATIONS.

Transudation fluids may be serous, sanious, or, in rare instances, chylous. Their sp. gr. is in general lower than that of inflammatory effusion into the same cavity. They are always alkaline,[49] and for the most part of a yellow colour.

Microscopically they exhibit but few tissue elements—fewer than, but in other respects similar to, those of serous exudation. It should be noted that in serous pleuritic effusion a large quantity of endothelium is often detached. This must not be taken to imply endothelial proliferation in a new growth (carcinoma), unless blood be also present, when such an inference acquires additional support. Chemically, transudation products exhibit much albumin, and generally sugar.[50] For the detection of the latter the process detailed at p. 48 may be employed. They are always free from peptone.

Such fluids are distinguished from exudations chiefly by their low sp. gr. and the difficulty with which they coagulate. The distinction, however, is not easy to make in many instances.[51]

The author would observe that in six specimens of transudation fluid and serous exudation, which were entirely free from blood-corpuscles and dissolved blood-pigment, he succeeded in separating no small quantity of urobilin. He is at present engaged in researches with the object of determining whether this body is a normal constituent of serous fluids. In the transudation from a case of cirrhosis of the liver *Moscatelli* recently found allantoin.[52]

C. CONTENTS OF CYSTS.

The physician has often to decide whether a particular specimen of fluid withdrawn from the body by aspiration or puncture is inflammatory in its origin or due to passive congestion, and finally whether it is derived from a cyst. Such a question is least apt to arise in exploration of the pleural cavity, but in connection with abdominal symptoms it is frequently very urgent to be able to give a definite answer. And this is not always easy; at times, indeed, it is impossible.

The cysts with which we have to do in this way are hydatid and ovarian cysts, and, in rare instances, cystic kidney.

1. Hydatid Cysts.—The fluid obtained by puncture from a hydatid cyst is clear, alkaline, and usually of low sp. gr., 1.006–1.010. It contains a small quantity of a reducing substance (grape-sugar), very little albumin, and abundance of inorganic salts, as sodium chloride.[53] Succinic acid and inosite have also been detected. The microscopical appearances are very characteristic. Amongst these are the hooklets (see also p. 80) and portions of echinococcus membrane, so readily distinguished by its transverse striation and uniformly granular inner surface (p. 80, fig. 45). Scolices may also be seen, and are known by the two circles of hooklets and four suctorial discs on the anterior aspect (head) and the sack-like hinder part, which is separated from the head by an annular constriction. If the cysts have suppurated or be filled with blood, as sometimes happens, chemical examination will usually throw but little light upon its character. An absolute diagnosis is possible only when hooklets or shreds of membrane have been seen in the fluid. Consequently it is well to receive the latter in a conical glass, and to carefully examine the sediment for these bodies.

Hydatid cysts often contain hæmatoidin crystals.

2. Ovarian Cysts.—The fluid obtained from ovarian cysts is remarkably variable in its character.

It is generally to be distinguished from inflammatory and congestion fluids by its high sp. gr., which ranges from 1.020 to 1.026. Its reaction is alkaline, and it has little tendency to coagulate.

It is further marked by the great abundance of tissue *débris* which it contains, and, from the nature of the cells which preponderate, information may be drawn as to the kind of cyst from which it has been taken.

Instances, however, occur in which the fluid from an ovarian cyst shows nothing by which it may be known from that of ascites, and, exceptionally, it has a sp. gr. even lower than that of a transudation fluid. According to *Schatz*, *Gusserow*, and *Westphalen*,[54] a low sp. gr. with little albumin points to a cyst of the broad ligament.

When hæmorrhage has taken place into the cyst, its contents may vary in colour from red to a chocolate-brown, and be very turbid. The microscopical examination of ovarian fluid shows a very variable quantity of red and white blood-corpuscles, and many forms of epithelium,

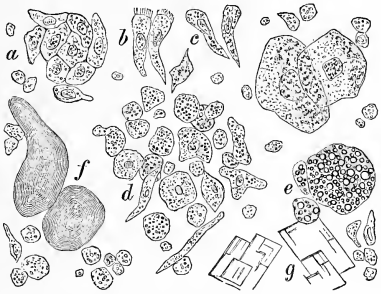


FIG. 123.—*a*. Squamous epithelium cells; *b*. Ciliated epithelium cells; *c*. Columnar epithelium cells; *d*. Various forms of epithelial cells; *e*. Fatty squamous epithelial cells; *f*. Colloid bodies; *g*. Cholesterin crystals.

squamous, columnar, and ciliated (fig. 123, *a*, *b*, *c*). These cells, however, are rarely well preserved, but are for the most part far advanced in fatty degeneration, and often with difficulty recognisable. Colloid concretions (fig. 123, *f*), in all probability derived from epithelium, are invariably found in the so-called colloid cysts.

Certain forms of ovarian cysts may be readily differentiated by a microscopical examination of their contents. Thus in dermoid cysts may be seen, besides squamous epithelium, hairs and crystals—cholesterin, fatty, and hæmatoidin. Chemical investigation, too, throws much light upon the character of an ovarian fluid. It will be found as a rule to contain albumin, and always metalbumin (paralbumin), and it is this body principally which renders it turbid and stringy (*Hammarsten*).[55]

To Test for Metalbumin.—The fluid is mixed with three times its

bulk of alcohol and allowed to stand for twenty-four hours, when it is filtered, the precipitate squeezed out, and suspended in water, which is again filtered. The filtrate is opalescent and has the following characters:—(*a.*) On boiling it becomes turbid, but does not form a precipitate. (*b.*) No precipitate forms with acetic acid. (*c.*) Acetic acid and ferro-cyanide of potassium render the fluid thick and impart to it a yellow tint. (*d.*) Millon's reagent and boiling yield a bluish-red colour. (*e.*) Concentrated sulphuric and glacial acetic acid yield a violet colour (*Adamkiewicz*). (*f.*) *Huppert*[56] has pointed out that when the fluid containing metalbumin is boiled with sulphuric acid it yields reducing substances, and this he regards as one of its most characteristic properties. It must be noted that metalbumin is a constituent of other pathological fluids besides that of an ovarian cyst. These cysts, and especially dermoid cysts, also hold a considerable quantity of cholesterin in solution. Cystic fluids have been frequently shown to contain diastatic ferment in appreciable quantity.[57]

3. Cystic Kidney.—In all cases where a sufficient specimen can be obtained, the fluid from a cystic kidney (hydronephrosis) may be at once identified on the ground of its chemical properties and microscopical appearances. An important point is the presence of renal epithelium, and this should be carefully sought for. Then the determination of urea and uric acid in large quantity indicates a connection with the kidney; but it must not be forgotten that these bodies occur more or less plentifully in ovarian cysts, or may be supplied to them when they communicate with the urinary tract by an abnormal channel.

It has been said that for the purpose of diagnosis the greatest significance attaches to the presence of epithelium from the urinary tubules; and inasmuch as this usually occurs very sparingly in the fluid from a cyst, the latter after removal by puncture should be allowed to settle and the sediment carefully examined. Such of the clinical symptoms of cystic kidney as call for notice here are very various. *P. Wagner*[58] observed that the urine is often scanty. Albuminuria and intermittent hæmaturia, symptoms of sufficiently vague import, also occur.

D. THE SECRETIONS FROM FISTULÆ.

Where these consist of a purulent, or simply serous fluid, discharging through an unnatural outlet, their diagnosis may be based upon the considerations already dealt with under the headings Transudations and Exudations. A greater, or at least, at present, a more physiological interest attaches to the appearances in those cases where fluids are discharged by an unnatural opening, which might be assumed to

communicate with the intestine, because the fluid closely resembles the intestinal fluid in its physiological properties, while fuller investigation shows that it is derived from cavities lined by secreting glandular epithelium.

The author has analysed the secretion from a case of this kind which *Professor Wölfler* observed and operated upon.[59] The fluid had an acid reaction, and contained albumose and peptone in considerable quantity, pepsin, and a ferment which changed maltose into grape-sugar; no diastatic ferment.

The maltose employed in this research gave Trommer's reaction only very faintly (see p. 225). When it acted upon the secretion at 40° C., both Trommer's and Nylander's tests gave positive results.

Small quantities of free hydrochloric acid were perhaps also present —the Congo-red and benzo-purpurin tests gave feeble results—but there was no sugar, urea, bile-pigment, or urobilin. Of inorganic salts there were chlorides.

From this statement it appears that the fluid in many of its characters resembled the mixed secretion of the intestinal tract.

CHAPTER IX.

THE SECRETIONS OF THE GENITAL ORGANS.

I. THE SEMINAL FLUID.

1. Naked-Eye Appearances of the Semen.—The seminal fluid is a thick, white, and somewhat opaque fluid, of slightly alkaline reaction. It is tolerably tenacious, and resists the pressure of the cover-glass. It owes this property to the presence in it of a gelatinous substance, which, under the microscope, appears to be hyaline, and encloses innumerable cavities of various sizes. The semen has a peculiar odour, which is derived, according to *Fürbringer*,[1] from the prostatic fluid, and depends upon the large proportion of compounds of Schreiner's base (æthylenimin) (see p. 81) which it contains.

2. Microscopical Examination of the Semen.—The spermatozoa, which are to be seen in great numbers in the semen, are thread-like bodies about 50 μ in length, and provided with a head 4.5 μ long, and compressed in one plane, so as to appear club-shaped when seen from the side. They exhibit very lively movements, but their motility is rapidly destroyed by the addition of water, drying, &c. They are present only in semen and in fluids with which the latter is mixed, and obviously they may possess a great interest in the diagnosis of certain morbid conditions. It may happen that the physician will have to examine the semen to settle a question of sterility. A persistent absence of spermatozoa (azoospermia) will show that the individual is incapable of procreation, and this may occur whilst the other signs of sexual power are retained. Of forty cases of sterile marriages, *Kehrer*[2] found that azoospermia was the cause in fourteen. It is very important to distinguish the persistent condition from the temporary absence of spermatozoa, which occurs as a result of excessive and repeated intercourse. Under such circumstances the fluid ejaculated consists almost entirely of prostatic secretion (*Fürbringer*).[3]

In addition to spermatozoa, the semen exhibits certain cells—large and small, finely granular testicle-cells, with one or more nuclei—some columnar and squamous epithelium, a few large round hyaline bodies, lecithin corpuscles, and stratified masses of amyloid substance, which

are finely granular within, and usually enclose a central kernel—these are derived from the prostatic solution ; and, finally, a few leucocytes—which usually have two nuclei—and spermatic crystals. Some red blood-corpuscles may also be seen.

Certain pathogenic micro-organisms, and especially tubercle bacillus, may be present in the secretion from the genital tract. They are usually discharged with the urine. The seat of the disease may be determined in such cases by other signs, as swelling of the testicle and epididymis, &c. (comp. p. 192).

In certain pathological conditions the semen may be coloured a chocolate-brown from the presence of a quantity of amorphous blood-pigment. This happens especially in old people and in persons who have often suffered from orchitis.

A very particular interest attaches to the spermatic crystals. In their appearance and chemical properties they very closely resemble

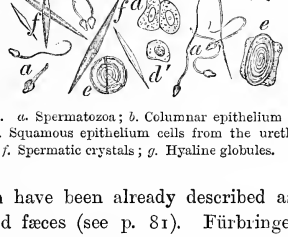


Fig. 124.—Seminal Fluid. *a*. Spermatozoa; *b*. Columnar epithelium cells; *c*. Bodies enclosing lecithin granules; *d*. Squamous epithelium cells from the urethra; *d'*. Testicle cells; *e*. Amyloid corpuscles; *f*. Spermatic crystals; *g*. Hyaline globules.

the crystals which have been already described as occurring in the blood, sputum, and fæces (see p. 81). Fürbringer has shown that while the basic compound is derived from the prostatic fluid, the phosphoric acid combined with it is furnished by the other component of the semen—the spermatic or testicular secretion. The crystals form immediately and in great abundance, when a 1 per cent. solution of ammonium phosphate ($[NH_4]_2HPO_4$) is added to the pure prostatic fluid, and their presence in large numbers, therefore, under all circumstances, indicates prostatorrhœa (see p. 306).

It follows, therefore, that such crystals are not characteristic of the semen, and the admixture of that secretion with some fluid or in a dried discharge can be established only on the discovery of spermatozoa. For this purpose, when dried, they must be dissolved out in water from the discharge containing them.

3. Chemical Examination of the Semen.—But little information can be derived in this way. According to Miescher, the funda-

mental constituent of spermatozoa is nuclein. Globulin and serum-albumin have been found in the semen, and it is very rich in inorganic substances. *Posner*[4] asserts that albumose is also present.

II. SECRETIONS OF THE SEXUAL ORGANS OF THE FEMALE.

1. Mammary Secretion (the Milk).—During the entire period of gestation, and especially from the third month of pregnancy onwards, a thin, whitish, and more or less turbid fluid may be obtained by pressure from the breast. This is a fact of great importance, and the existence of such a secretion is by itself strong evidence of pregnancy.

The fluid in question, when examined microscopically, presents in the first place a great number of strongly refractive bodies of very irregular size. These are called colostrum corpuscles; they are fatty in character, and are usually aggregated in groups. In addition to these are to be seen a few leucocytes and some epithelial cells from the inner surface of the ducts.

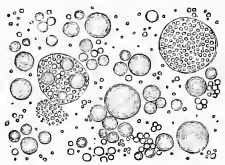


FIG. 125.—Colostrum.

Immediately after confinement the colostrum corpuscles disappear from the milk, and eight or ten days after parturition are no longer to be found. In their place is seen a profusion of fatty globules of irregular dimensions, and together with these certain particles (*Hoppe-Seyler*) which consist of casein and nuclein.

In diseases of the breast, and especially in cases of abscess and inflammation during suckling, the milk is apt to exhibit an intermixture of leucocytes.

Micro-organisms occur in the secretion in connection with certain morbid states. Thus *Escherich*[5] found fungi in the milk of a woman suffering from septicæmia, and these on cultivation proved to be pathogenic. Pathogenic Staphylococci have also been isolated by Koch's cultivation-process from the milk of a woman with facial erysipelas (*Karlinski*).[6] No other cases have been recorded where pathogenic fungi have been found in human milk.

From observations which the author has made, it would seem pro-

bable that tubercle bacillus also is occasionally present. The matter deserves further notice.

The milk of some of the lower animals has been known to be infested by non-pathogenic micro-organisms (Bacillus cyanogenus and Micrococcus prodigiosus), from which it may derive an abnormal blue or reddish tint (*Neelsen, Hueppe*).[7]

The chemical constitution of the milk varies under different conditions both of health and disease. The milk of sick women is generally less rich in fat, and the proportion of lactose is diminished. Neither bile-pigments nor biliary acid have yet been satisfactorily demonstrated in the secretion of jaundice (*v. Jaksch*).[8] The albuminous constituents of human milk are serum-albumin, casein, and nuclein. It also contains milk-sugar and fats. For the detection and estimation of the latter, the method described for the same purpose in the chapter on urine may be employed. Special processes for the

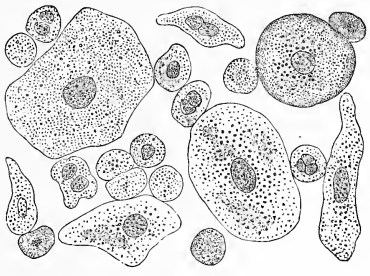


Fig. 126.—Cancer-Cells from the Vaginal Secretion.

quantitative analysis of the milk may be found in Hoppe-Seyler's work.

The examination of the milk of wet-nurses is a point of practical interest for the physician. It should be tested carefully as to its naked-eye and microscopical characters, and in all cases chemically analysed. In addition to this, information may be derived from the employment of the bacteriological methods. And it is highly expedient that the milk, whether of healthy or diseased women, should be submitted, wherever possible, to Koch's plate-cultivation processes, so as to ascertain the absence of fungi.

2. Vaginal Secretion.—This, under ordinary circumstances, is a thin fluid with an acid reaction. It contains a few large leucocytes, each with a single nucleus, and squamous epithelium cells, which, for the most part, are covered with microbes. In vaginal catarrh the

number of leucocytes is greatly increased, and some red blood-corpuscles may be visible.

In cases of ulcerating carcinoma implicating the vagina or the vaginal portion of the uterus, a copious fœtid discharge takes place, and in this the characteristic large cells of cancer may be found (fig. 126).

Hausmann[9] detected fatty needles in the vaginal mucus.

Among the parasites which have been found in this situation are :—

1. Yeast and Fission-Fungi.—Various fungi belonging to these classes infest the vagina. Thrush-fungus vegetations also have been seen there. The vaginal secretion normally (*Winter*[10]) and during confinement (*Döderlein*[11]) contains fission-fungi, as, *e.g.*, Staphylococcus pyogenes albus, aureus, and citreus. Finally, it is sometimes important to examine the secretion for tubercle bacillus and gonococci by the prescribed methods.

2. Trichomonas Vaginalis.—This parasite is an infusorium of oval shape, and about 10 μ in length. It has a long caudal appendage, three flagella, and a lateral row of cilia.

Of the chemical constitution of the vaginal mucus little is known. It has been said to contain trimethylamin (*Hilger*).[12]

3. The Uterine Secretion.

1. Menstruation.—At the outset of menstruation there is an increased discharge of vaginal secretion. Later on there is mixed with this an abundance of red blood-corpuscles and prismatic epithelial cells laden with fat from the interior of the uterus. The proportion of blood-cells begins to diminish after a few days, and leucocytes preponderate towards the close of the period. At this time also epithelium and a large quantity of fatty detritus are discharged.

2. The Lochia.—The fluid discharged during the first few days after parturition is thin and of a red colour. In addition to red and white blood-corpuscles it exhibits uterine and vaginal epithelium. Later, whilst the red corpuscles diminish in number, the leucocytes and epithelium increase, and the discharge assumes a grey or even white colour.[13] Microbes are always plentifully present, even in the absence of septicæmia. According to *Döderlein*,[14] healthy lochia are free from fungi, while in disease he found them without exception to contain Streptococcus pyogenes.

A point of special importance in diagnosis is the examination of the uterine secretion for the pathogenic fungi, to which reference has been already made. The secretion may be removed for the purpose by means of a tampon.

CHAPTER X.

METHODS OF BACTERIOLOGICAL RESEARCH.

The great practical importance which this subject has acquired of late makes it necessary that the physician should be familiar with the processes for ascertaining the presence of micro-organisms.

In all cases where micro-organisms may be the exciting cause of disease, his first task will be to detect them in the secretions or natural fluids by means of the appropriate methods for staining them.

When this is done, it remains in many instances to discover the micro-organisms in particular situations, cells or tissues, so as to exclude a mere misleading coincidence.

Further, the micro-organisms are to be cultivated outside the system; so that, where their morphological character and their behaviour to staining substances are not sufficiently distinctive, the requisite inferences may be drawn from their mode of growth and development.

Finally, it is possible by experiments upon animals to settle definitely whether the transmission of a pure cultivation of the micro-organisms will produce symptoms more or less closely resembling those attributed to their agency in man.

The staining methods at our disposal and the perfection of our optical instruments in many cases render the detection of micro-organisms easy, but their cultivation and the transferring of them to animals is often very difficult. Thus in the case of many diseases, micro-organisms have been discovered under such circumstances as to leave no doubt that they were the exciting cause, while every effort towards their cultivation and transmission to animals has failed of success. This, however, has been achieved in the case of a number of pathogenic fungi, as, for instance, the bacilli of glanders, anthrax, tubercle, cholera, leprosy, and probably of typhoid fever.

It is no longer necessary for diagnosis to pursue the inquiry in every instance through its entire course (detection, cultivation, and transmission to animals); but in some diseases, as tuberculosis, it is sufficient to note the characteristic effect of staining substances. In others, again (as relapsing fever and, occasionally, anthrax), a simple microscopical examination will serve, without the application of staining

processes. In doubtful cases of anthrax, the diagnosis may be settled securely by the direct transmission of the blood to animals.

With reference to Asiatic cholera, the mere detection of the fungus in the stools is never sufficient, but it must be isolated by Koch's cultivation methods, when it will be recognised by its mode of growth. With the progress of science we may learn to know a definite fungus as the cause of each of the infectious diseases; but even then, when all the conditions indicated above have been complied with, our labour will not have ended. It will remain to extend our acquaintance with the life-history of the parasites, so as to determine the sources of nitrogen and of carbon and the inorganic salts upon which they depend for their growth. It is only when this is done that a secure foundation will be laid for a system of rational therapeutics.[1]

A short account will be given here of the methods employed in such researches. It will naturally begin with a description of the apparatus, and in the first place of the microscope.

I. THE MICROSCOPE.

The shape, size, and adjuncts of the body or stand of the microscope itself are in general of little moment. Habit will determine, in most cases, whether the tube used shall be worked by a screw or with the hand. For the examination of plate-cultivations, however, the former will be chosen. Neither is it essential that the stand should be jointed, *but it is absolutely necessary that it be faultless in its construction. It must also be capable of adjustment for use with the most powerful objectives, and with an Abbe's condenser or equivalent arrangement.*

The stage must be sufficiently large and firm, and the opening in it as large as possible, so that a plate-cultivation, for instance, may be inspected easily with a low power.

For bacteriological investigations, as has been already observed, an Abbe's or other condenser adjusted movably to the microscope-stand is needed. The principle of such an instrument is this: The rays of light reflected from the mirror of the microscope are passed through the principal axis of a lens interposed between the mirror and the objective in such a direction that they fall upon the object, which thus receives a cone of light as highly concentrated as possible. If narrow diaphragms be introduced between the mirror and the collecting lens, an illumination of the image is obtained similar to, but probably somewhat more intense than, that where narrow cylindrical diaphragms are used. The edges of the image are in all cases well defined, even in unstained preparations, and such a condenser may be employed with advantage for histological work. If the diaphragms be removed and

an open-condenser light consequently employed, the outlines disappear and become entirely indistinguishable (*Koch*).[2] Under these circumstances nothing else can be distinctly made out with uncoloured preparations. With stained preparations, however, it is far otherwise, and in this lies the valuable application of the open-condenser light as discovered by *Koch*. The outlines, in so far as they depend for their appearance on a distinction in the refractive properties of the object (corresponding to a structural difference amongst its parts) and those portions which are but lightly stained, are lost to view, while the deeply-stained particles, as the coloured nuclei of cells (granulations), and especially fungi coloured with aniline or other dyes, become more exquisitely defined. In this way micro-organisms may readily be seen and recognised, even when very sparsely present in a preparation. *The apparatus is indispensable for bacteriological research.**

In addition to a reliable stand and a condenser, good objectives are needed.

First a weak objective, magnifying about 60 to 80 diameters, for the inspection of plate-cultivations; and in addition it is very advantageous to have a good powerful dry objective. There are many objects, as fresh blood, fresh milk, or fresh pus, for the examination of which immersion lenses are not suitable. For such purposes Zeiss's lenses F or D, or Reichert's 8 A, may be recommended. With these, especially if a condenser be also used, many bacteriological preparations, as those of tubercle bacillus from the sputum, may be adequately investigated. For very delicate preparations, and in particular where minute details of structure are to be made out, an immersion system is needed. The water-immersion systems, formerly much in use, have been superseded of late by the oil-immersion (homogeneous immersion) lenses made by Stephenson and Abbe and Zeiss, which are to be preferred on account of the better definition and clearness of the image which they produce. Instead of water, there is interposed between the front lens of the objective and the object (cover slip), a fluid having the same refractive index as glass. For this purpose a mixture of fennel- and castor-oils may be used. Reichert employed with his lenses a mixture of vaseline and olive-oil, which has the advantage of being odourless, and of penetrating less readily within the lenses. At present concentrated cedar-oil is most in use. Such a system has the further advantage that it does not require correction-collars to be used, as do dry lenses, and that powerful eye-pieces may be employed with it. It is very

* The best results are obtained with the instruments supplied by *Hartnack* (Potsdam), *Seibert* and *Krafft* (Wetzlar), *Leitz* (Wetzlar), and especially by *Zeiss* (of Jena). *C. Reichert* of Vienna supplies with his little microscopes IV. and V. a condenser which is very suitable for clinical purposes.

advantageous also to place a drop of oil on the under surface of the slide which carries the object, between this and the collecting lens of the condenser.

Of less moment is the choice of an eye-piece. In general, for every form of investigation, except bacteriological research, eye-pieces of low magnifying power should be taken. For the rest, the eye-pieces II. and V. as supplied by the firms of Reichert and Zeiss will serve for all cases. The periscopic eye-pieces of Seibert and Krafft are very excellent.*

An objective of crown- and flint-glass was first adopted by Dr. Schott of Jena. It is an admirable contrivance, and has become indispensable for many purposes, as in photographing micro-organisms, where a well-defined achromatic outline is required. Zeiss has given such the name of apochromatic objectives. The corresponding compensation eye-pieces should be used with them. Their chief advantage lies in the fact that they give a clear and well-defined image with eye-pieces of greater strength than could formerly be used. Apochromatic objectives of excellent construction are supplied by Reichert. The images of the most delicate objects obtained with Reichert's homogeneous immersion objective of 2 mm. focal length, and even with the working eye-piece 12, are clear and distinct in their smallest details. There is one disadvantage attending the use of these lenses. They need a finer adjustment than the usual mechanism affords, and the image becomes indistinct with the slightest movement of the instrument. It has then to be focussed anew. This defect is recognised in the more recent microscopes of Seibert and Krafft, which carry a third very fine adjustment to remedy it. [See Appendix XXIV.]

II. THE DETECTION OF MICRO-ORGANISMS.

In many cases the object to be examined may be placed under the microscope without preparation of any sort. The characteristic micro-organisms will then become visible. This is so with the spirillum of relapsing fever, anthrax bacilli in the blood, &c. For the most part, however, special processes are necessary for the detection of micro-organisms. The details of some of the processes for the preparation of specimens have been gone into in the chapters on the *Blood*,

* The author has for many years employed an instrument made by Reichert, and he has found it to serve well in every kind of microscopical work, histological and bacteriological. Its parts are as follows:—Eye-pieces II. and IV., objectives 4, 8 A, and oil-immersion $\frac{1}{15}$; a small stand with condenser (Abbe's) and cylinder diaphragm. Its price was 207 florins without the oil-immersion, which cost 107 florins. Very good and inexpensive systems of lenses are also made by Plössl of Vienna.

Sputum, &c., which the reader will consult for information concerning them.

It will not be out of place if we give here a brief summary of the methods in use, and point out the particular purposes to which each is applicable. The principles upon which all of them are based were worked out by Koch, Ehrlich, and Weigert; and almost every day some new process or a modification of the old familiar methods is made known. It would far exceed the limits of this book to attempt an account of all the methods which have been suggested and the various modes of applying them. We shall confine ourselves to what has appeared in the more important and systematic essays dealing with the subject. Those of *Günther*[3] and *Unna*,[4] especially the latter, comprise an accurate and exhaustive account of the methods for staining fungi. Particularly good results in the staining of micro-organisms in sections, which, however, lies beyond our province, have been obtained with *Kühne's*[5] processes. They have been tested by Rille, a pupil of the author, and have been found most valuable for dry cover-glass preparations as well. The methylene-blue method[6] and *Kühne's*[7] modification of Gram's method (staining with alcoholic solution of Victoria-blue) are very serviceable in the examination both of sections and of the secretions.

In the examination of the blood and secretions for pathogenic micro-organisms it is in general best to proceed with the use of basic aniline dyes as described at p. 27. Should it happen that no result is obtained in this way, greater certainty may be secured with *Löffler's* method (p. 28), which is especially suitable for detecting the bacilli of typhoid fever and glanders, and, finally, with Gram's method (p. 28). All the fungi hitherto discovered stain with this, *except the bacilli of typhoid and cholera*, and *gonococci*. The bacillus of hen-cholera is also unaffected by it.

Günther's method (p. 31) is very serviceable for staining the spirillum of relapsing fever. The investigation of the blood and secretions for tubercle bacillus should be carried out precisely in the manner laid down by Koch and Ehrlich (p. 75). Staining with basic aniline dyes serves well for the detection of fungi in the buccal cavity, the nasal secretion, and the gastric contents; but for examining the buccal secretion Gram's or Günther's method may also be adopted with advantage, since they render visible the very delicate Spirochæta buccalis (p. 55 and capsulo-cocci.

In searching for the fungi of the alimentary canal, pathogenic and non-pathogenic, all the processes hitherto mentioned should be employed where a thorough investigation is aimed at, and the observer should not forget to add a little iodo-potassic-iodide solution to a drop of the fluid under examination (see p. 131).

In examining the urine, the best results are secured with Gram's or Friedländer's method (p. 78). By their aid the author has detected in various specimens of urine from persons both in health and disease an unexpected profusion of different forms of fission-fungi.

The micro-organisms which occur in pus stain most readily with Gram's method, or its modification already referred to (Victoria-blue). Löffler's and Friedländer's methods are applicable to the same purpose.

To stain the spores of micro-organisms a preparation is made in the manner indicated at p. 27, but exposed for a longer time to the heat. It may be passed through the flame about ten times (*Hueppe*).[8] When this is done the bacilli lose their staining properties, whilst the spherical objects, if they consist of spores, take up colouring-matter. It is still better to employ the process of double staining. The preparation is first stained in a hot Ziehl-Neelsen fuchsin solution, then decolorised with nitric acid, and again stained with methylene-blue. The spores then appear red and the bacilli blue.

III. CULTIVATION OF MICRO-ORGANISMS.

A. Methods of Sterilisation.—When the presence of micro-organisms has been ascertained with certainty by the processes described above, the next task is to secure their development outside the system, in other words, to cultivate them. To this end the first requisite is the means of sterilisation. *The essential condition that must be secured in all such cultivation researches is the absolute freedom of the instruments and vessels employed from fungi and germs capable of development.*

In the case of metallic instruments, the necessary cleanliness may be attained best and most readily by raising them to a red heat in the flame of a Bunsen's burner. Glass vessels, such as test-tubes, flasks, &c., should be purified as far as possible by washing first with distilled water, then with corrosive sublimate solution (1 : 1000), and rinsing out with alcohol and æther, after which they may be sterilised in a dry heat. This can be done best by a sterilising apparatus for temperatures over 200° C.; but if such an apparatus cannot be had, it may also be done by heating the vessels cautiously over the flame of a Bunsen's burner. In the latter case they should first be carefully dried, in order to prevent the glass from cracking, and the mouth of each should be closed with a compact plug of sterilised cotton-wool before heat is applied.

It is very important to heat the sterilised vessel again immediately before using it, having previously ascertained that the plug is at once sufficiently compact and easily extracted.

The plug may be made of fine glass-fibre, or, still better, of asbestos, instead of cotton-wool.

Test-tubes which are to be held in readiness for use are purified in the manner described, fitted with a plug, and then placed in wire baskets, and sterilised in a dry heat.

The sterilisation of the nutrient fluids presently to be mentioned may be effected by boiling them in glass flasks furnished with a plug of cotton-wool in those cases where the constitution of the fluid is not altered by heat. To sterilise nutrient gelatine and agar-agar solution (see p. 320), these substances are repeatedly boiled in the vapour sterilisation apparatus. Too frequent, and especially too continued, boiling should be avoided in the case of these two substances, lest they should remain fluid after cooling.

When potatoes are employed as the nutrient substance, they are first freed from sand with a brush, placed for an hour in a 5 per cent. corrosive sublimate solution, finally sterilised (boiled) by steam at boiling-point, and cut into strips with a sterilised knife. Where the vapour sterilisation apparatus devised by Koch cannot be had, a Papin's digester with a perforated tray will serve. It is more difficult to sterilise those nutrient substances that will not bear a heat of 100° C. without their parts coagulating and so becoming opaque. In their case, Koch recommends that they should be sterilised by intermittent heating. This plan is especially useful for the purpose of freeing blood-serum from fungi and germs.

To prepare sterilised blood-serum, Koch proceeds as follows:—The part of the animal's skin through which the blood is to be taken is shaved, and thoroughly cleansed by washing it with solution of corrosive sublimate, alcohol, and æther. The underlying blood-vessel is freely exposed, and opened with sterilised instruments. The blood is then made to flow directly from the artery into a sterilised glass cylinder, which is filled to the brim and placed in a refrigerator or upon ice for twenty-four to forty-eight hours, so as to allow the corpuscles to settle. The clear amber-coloured serum that has separated after twenty-four hours is drawn off in sterilised pipettes and placed in test-tubes previously sterilised in the manner already indicated. These are heated to 58° C. for two to six hours, and finally the serum is made to coagulate by heating it to 65°–68° C.

With a view to obtaining the largest possible inoculation surface, it is desirable to procure the coagulation of the fluid in the test-tube with its surface inclined very obliquely to the latter. This end may be attained by using a tin vessel with double walls between which water may be retained, covered on top with a plate of glass, and having at its anterior end two movable feet which can be fixed by screws. Or instead of this the test-tube may be fixed by a clamp at an oblique angle in a pot filled with water. For many purposes, and especially

for the cultivation of the pathogenic fungi occurring in man, human blood-serum should be employed. To procure human serum the author has adopted the following plan :—The skin is first thoroughly cleansed in the manner already described, and a puncture is made in it with a cupping-blade previously sterilised by exposure to a heat of 200° C. The blood is taken in a cupping-glass similarly sterilised, and poured into small test-tubes also sterilised. The remainder of the process is carried out as before. Human serum has proved, in the author's experience, to possess notable advantages over that of animals. It remains clearer after coagulation, and is firmer. Where human blood-serum cannot be had, transudation fluid or serous exudation may be taken instead. In that case it is prepared in the same way as the former. A very useful modification of the process for the preparation of blood-serum and of blood-serum plates has been devised by *Unna*.[9] To the blood-serum of the calf, peroxide of hydrogen is added drop by drop, until the mixture, which was at first a brownish-yellow, becomes clear. For this purpose a quantity equal to about half the volume of the serum will be required. The mixture is neutralised with a 2 per cent. solution of sodium carbonate and passed through a wet filter packed to one-fourth of its depth with well-calcined diatomaceous (Kieselguhr) earth. The fluid which first passes through is usually turbid, and must be refiltered; and, finally, the clear filtrate is sterilised in the usual manner. For making plate-cultivations Unna recommends the admixture of 10 per cent. gelatine or 6 per cent. agar-agar.

B. Nutrient Substances.—By the methods described at p. 314 we are enabled to detect micro-organisms; and the measures that must be taken to render the fluids and nutrient substances as well as the instruments employed in research free from fungi have also been indicated.

It does not suffice to place a fungus or the germs of fungi at random in solid or fluid nutrient substance duly sterilised in order to ensure their successful cultivation; but there is for each of the pathogenic and non-pathogenic fungi an appropriate soil, which differs widely in its chemical constitution in different cases. The researches of *Pasteur*[10] in connection with yeasts, of *Nägeli*[11] and *Buchner*[12] with bacteria and moulds, of *A. Schultz*[13] with moulds, of the author[14] with the micrococcus ureæ, and of *Hueppe*[15] with the lactic acid bacillus, have shown that, in addition to a supply of nitrogen and carbon, each fungus requires certain inorganic salts for its growth. And further, there is in each case some particular temperature at which the fungus thrives best (optimum temperature).

It is only when all these conditions have been secured that cultivations can be made successfully.

To draw unequivocal conclusions from researches of this sort, it is above all necessary to apply Koch's process, presently to be described, for obtaining pure cultivations of the fungus which is being investigated, and to implant these upon solid or fluid nutrient substances.

1. Nutrient Fluids.—The use of fluids as nutrient substances entails uncertainty in the result, since the microscope cannot be employed to examine them. Still it is not difficult to obtain a pure cultivation in a fluid if it be sterilised, and if a cultivation known to be pure be planted in it.

The process is then the same as for the preparation of pure cultivations by Koch's method, which will be described presently.

The composition of the nutrient fluid must vary with the nature of the fungus to be cultivated.

Thus yeasts grow best in a somewhat acid saccharine fluid. Moulds require a medium holding free acids in considerable quantity. Feebly alkaline fluids are the best for certain non-pathogenic bacteria. Fluids of definite constitution for the cultivation of bacteria—as those of Pasteur, Cohn, and the author—agree in that they all contain nitrogenous and carbonaceous matter and inorganic salts.

Although it is true that by cultivation in nutrient fluids much useful information has been obtained concerning the life-history of some of the fission-fungi, the method is not employed for the investigation of pathogenic micro-organisms, partly because, as has been said already, there always remains some doubt as to whether the cultivations obtained with them are in reality pure cultivations, and partly because it would seem that pathogenic fungi thrive badly in fluids. The author has endeavoured, without success, to obtain pure cultivations of pneumonococci, Streptococcus pyogenes aureus, and other pathogenic micro-organisms, on sterilised nutrient fluids of the most varied constitution.

Control experiments have proved that non-pathogenic fungi developed readily in such nutrient fluids, while the same fluids, maintained under like conditions, when infected with pathogenic fungi remained sterile.[16]

2. Solid Nutrient Substances.—As in the case of nutrient fluids, the chemical constitution of solid nutrient substances will vary greatly according to the biological character of the fungus under cultivation.

1. Blood-Serum.—The blood-serum of animals is required for certain pathogenic fungi, *e.g.*, tubercle bacillus; gonococci are cultivated in human serum. The method of preparation has been sufficiently described.

2. R. Koch's Nutrient Gelatine.—This is prepared in the following manner:—500 grms. of good meat, free from fat and freshly minced,

is rubbed up with 1000 grms. of distilled water and placed for twenty-four hours in a refrigerator. It is then strained through linen, the resulting fluid made up to 1000 cc., and 10 grms. of dry peptone, 5 grms. of common salt, and 100 grms. of white edible gelatine are added. The fluid is heated in a flask until the gelatine is dissolved. It is then accurately neutralised with sodium carbonate, and boiled for half an hour to an hour, when a specimen is tested for its reaction; after this the fluid is filtered through a hot-water funnel and poured into test-tubes sterilised in the manner directed (see p. 317), and sterilised for ten minutes a day for three days.

The test-tubes may now be kept for weeks or months at the ordinary temperature before they are used; but when this is done, an india-rubber cap should be placed over each above the plug of cotton-wool, to prevent evaporation from the gelatine. Keeping the nutrient gelatine thus for a long time in the test-tubes has this advantage, that when through an accident in its preparation germs have obtained admission to the fluid, the resulting cultivations are made evident by clouding in the gelatine, which will not then be employed for cultivation research.

Koch's nutrient gelatine, which may be modified at will by the addition of organic or inorganic substances, will serve for the cultivation of all pathogenic and non-pathogenic fungi which grow at the ordinary temperature of a room. It cannot be used at higher temperatures (over 25°–30° C.), which cause it to melt, and for micro-organisms which liquefy gelatine rapidly.

3. **Agar-Agar.**—In many investigations, especially for fungi which require a temperature not lower than blood-heat to develop well, or which liquefy gelatine rapidly, agar-agar may be used with advantage instead of gelatine as a nutrient substance. It is prepared in the same way as nutrient gelatine fluid, only that instead of gelatine 1.5–2 per cent. of finely divided agar-agar is added to the fluid. Unfortunately it is difficult to prepare a perfectly pure and clear agar-agar solution in this way, and that substance, even when added in small particles, filters badly through the hot-water funnel. *Schottelius*[17] and *Richter*,[18] however, have modified the process in such a manner that a clear nutrient agar-agar may be easily obtained.

4. **Potato.**—Enough has been said about the sterilisation of potatoes employed for nutrient material (p. 317). Their use is of great service in studying pathogenic fungi, many of which develop on potato in a highly characteristic manner (see pp. 136, 295).

Starch to which suitable nutrient salts have been added forms a solid nutrient substance, which is very useful and easily sterilised. Together with gluten and bread it is especially good for the cultivation

of moulds. For the latter also boiled blood clots may be employed with advantage. Both starch and blood may be easily and effectually sterilised by a current of steam.

Recent investigators have in many instances somewhat modified the constitution of, and the method of preparing, nutrient substances. Thus the addition of glycerine to peptone gelatine or agar-agar has been tried successfully. All the modifications suggested cannot be noticed here. The use of stained nutrient solids and fluids, however, demands notice.

According to *Birch-Hirschfeld*,[19] living anthrax bacilli can be procured stained by inoculating from a pure cultivation of the fungus upon 15 per cent. nutrient gelatine which contains in 6 cc. 1 cc. of a watery solution of fuchsin or methylene-blue. The cultivation should remain for twenty-eight hours at 35°–40° C.[20]

In the cultivation of the bacillus of typhoid fever also, stained (benzo-purpurin) nutrient substances are useful.

The application of this principle, as by the addition of neutral tincture of litmus,[21] or other substances, to show the formation of free acid or acid salts, has thrown much light upon the nature of pathogenic and non-pathogenic fungi. The addition of a little watery solution of Congo-red or benzo-purpurin to nutrient substances has proved, in the author's hands, a valuable expedient in the study of the biological character of micro-organisms. If the material examined contains acid-forming fungi, the cultivations upon stained nutrient soil take a colour ranging from a pale to a blackish blue, and their presence is discernible with the naked eye. The method is particularly appropriate for the study of micro-organisms from the intestine under normal and pathological conditions.

C. Preparation of Koch's Pure Cultivations. — Although *Klebs*[22] and *Brefeld*[23] had already suggested, and themelves adopted, the use of solid nutrient substances for the investigation of fungi, it was *Koch* who grasped the significance of these methods, and, by submitting the cultivations in fluid and solid media to the control of the microscope, laid the foundation of modern bacteriology.

To this observer we owe not only the knowledge of many fundamental facts in bacteriology, such as result from the discovery of the bacillus of tubercle and of cholera, but nearly all the cultivation and staining methods in use are derived from his researches and those of his pupils.

The methods now to be described have for their object as far as possible to separate the individual germs from a mass of fungi, and to promote their development apart from one another in solidifying fluids. This may be done by Koch's processes for obtaining plate-cultivations

on glass slides and test-tube cultivations. It is usually expedient to make plate and test-tube (deep inoculation) cultivations together.

1. Plate-Cultivations.—A test-tube, in which from 5–8 cc. of solidified nutrient gelatine has been placed as directed above, is put into warm water, and allowed to remain there until the gelatine is quite fluid. Care is now taken that the plug in the mouth of the test-tube is freely movable by rotating it a little if necessary. The test-tube is held obliquely between the thumb and index-finger of the left hand, the plug with the upper end between the second and third fingers (Koch's "grasp"). A little of the fungoid material is taken with a freshly-sterilised platinum needle, and placed on the gelatine, so as first to touch its edge and then to mix with the fluid. In doing this, draughts of air must be excluded from the room. In the same way one or more drops of this first dilution are placed in nutrient gelatine in another test-tube (second dilution), and if a provisional inspection has shown that the fluid under examination is very rich in fungi, the process is repeated again (third dilution). It may then be assumed that the germs in the nutrient gelatine are actually isolated. The infected gelatine is next poured out on to glass plates about 14 cm. in length and 12 cm. broad, and caused to solidify quickly. This may be done in a few minutes by the application of cold. The glass plates are prepared in the following manner:—They are first thoroughly cleansed with water, solution of corrosive sublimate, and alcohol, and placed immediately before use in iron cages in the steriliser, where they are heated for a long time at 100°–150° C. They are taken out when cool, and laid upon a large sheet of plate-glass made cold with ice. This should have a polished, even surface, and must also be accurately level when used, an end best attained by mounting it on a tripod stand with a spirit-level visible beneath the glass. Such an accessory, however, is not essential, and it may be dispensed with if a little care be taken.

Recently the use of the glass plate cooled on ice has been supplanted in many laboratories by that of a polished plate of iron about 20 cm. in diameter and 8 cm. thick, which is carefully sterilised beforehand. This is placed upon the tripod and adjusted with the spirit-level. An iron plate was first employed in Lichtheim's laboratory at Berne, and it is very suitable to the purpose in hand, because with it nutrient fluids solidify very quickly. In warm weather, as in the summer, the iron plate must be previously cooled on ice, but ordinarily this precaution is unnecessary. The author has employed such a plate exclusively in his bacteriological work during the last four years, and found it most serviceable.

The nutrient gelatine is poured upon the plates in the following manner:—The small glass plates upon which nutrient gelatine is to be

spread are laid upon the glass plate made cold with ice or on the iron plate, and that edge of the test-tube over which the gelatine is to flow is heated. When the edge has cooled, the gelatine is poured here and there over the cold surface of the plates, and spread out upon them as evenly as possible by means of the sterilised edge of the test-tube, care being taken that the edges of the plates are left free. The plates are then covered with a bell-glass. When solidification is complete, they are placed on sterilised wet blotting-paper in a glass dish of about 30 cm. diameter, which has been previously well purified with corrosive sublimate, and another bell-glass is put over them.

In order to obtain the sterilised and wet blotting-paper, a jet of super-heated steam is directed upon the filter-paper in the bottom of the glass dish for from eight to ten minutes. In this way the bell-jar as well as the filter-paper are sterilised, and the latter is saturated with sterilised vapour.

In such a vessel six plates, or even more, can be put, with a glass partition between each pair.

Instead of plates, *E. Esmarch* [24] has recently employed test-tubes, which will serve for many of the purposes of plate-cultivations. With these the process is as follows. Some of the fungoid material is placed in the fluid nutrient gelatine in the test-tube in the manner already described, and mixed with it as intimately as possible. The test-tube, covered with an india-rubber cap above the plug of cotton-wool, is then held as vertically as possible, and with the sterilised plug and cap directed somewhat upwards, under a stream of cold water, and in this position is made to rotate upon its long axis. After a little while the nutrient gelatine has solidified in the cylindrical form of the test-tube. This process has considerable advantages. Cultivations so made can be inspected not only with weak (Reichert IV.), but also with powerful objectives; they are less likely to be contaminated; individual cultivations may be removed with a little care even under the microscope, and less annoyance is apt to arise from the unpleasant odour which the cultivations often give rise to than is the case when plates are used. To these the name of cylinder-cultivations may be appropriately given. On a plate or in a test-tube so prepared, after a longer or a shorter interval, there appear little punctiform colonies, which are already sufficiently distinguishable from one another, and at the same time the gelatine is often partly liquefied and gives off a disagreeable smell. If now a minute particle be taken from each of the colonies by means of a sterilised platinum needle and submitted to the same process over again, pure cultivations will be had of all the fungi which develop in nutrient gelatine.

At the same time, by placing the entire plate under the microscope

and examining it, it will be possible to study the mode of growth of the fungi, and also to determine whether in any given case the cultivations are pure or contaminated by admixture with other fungi. Certain distinctions in the shape and colour of the cultivations can also be recognised with the naked eye, and by removing a particle on a platinum needle from an individual developing cultivation under the microscope and transferring it directly to a test-tube (cultivation by deep inoculation), a definite fungus may be cultivated in a little time and with little trouble.

Cultivations with nutrient agar-agar are made in the same way as with nutrient gelatine. The former substance should be used in all cases of fungi which contain spores and where the growing fungi cause nutrient gelatine to liquefy quickly; as, for instance, in making cultivations from the fæces, and in cultivating micro-organisms which require a high temperature (37° and upwards) for their growth.

The cultivations are placed in an incubator such as Koch and others (d'Arsonval) have devised. The construction of these is the same in all cases. The incubator has double walls enclosing a compartment for water, with arrangements (thermostats) by which the internal temperature may be maintained constant to within 0.2° C. Experience, and especially Koch's researches, have shown that a number of pathogenic fungi, as, for instance, the bacillus of tubercle, will thrive only at a definite and continuous temperature. To aid in securing this, various thermostats have been constructed recently. Of these, the author uses the thermo-regulator of *L. Meyer*.[25] The principle of the instrument is to regulate the supply of gas for heating the incubator by conducting it through an æthereal atmosphere confined by a mercurial valve, so that more or less gas is conducted to the jet according to the temperature required in the incubator.

This instrument answers well. Such a thermostat has been in use for several months in Professor Nothnagel's clinic. Notwithstanding the great variation in gas-pressure, the temperature recorded never varied by more than 0.2° C.

2. Cultivation by Deep Inoculation.—A particle of the fungoid mass is taken on a sterilised platinum needle and implanted on nutrient gelatine or agar-agar in a test-tube by removing the sterilised plug with the mouth of the tube downwards and plunging the inoculating needle into the nutrient substance. After the lapse of a few days the fungus develops in a very characteristic manner in the gelatine. This proceeding is adopted only when a pure cultivation has been obtained with plates, and it is then of service in the further study of the micro-organism. *R. Fischl*[26] has removed the cylinder of gelatine from the test-tube with a cork-borer, and *Neisser*[27] by the action of heat;

and by subsequently hardening it in alcohol or a 1 per cent. solution of bichromate of potash, these observers were able to observe the development of the fungus in microscopical sections.

3. Cultivations on Glass Slides.—A sterilised platinum needle is infected with a particle from the fungoid fluid in the manner indicated on p. 322, and drawn across the surface of nutrient gelatine spread upon a slide in such a manner that the fungi lodge in the furrow. After a few days colonies of fungi develop in the line of inoculation.

4. Cultivation in Hanging Drops.—Koch was the first to adopt this method of cultivation. With it the growth of the micro-organisms can be observed directly under the microscope. It is carried out thus:—A glass slide with a hollowed surface is taken, and the edge of the concavity is smeared with a little vaseline, or a mixture of five parts of vaseline and one of paraffin may be used instead, as Birch-Hirschfeld recommends. A drop of sterilised broth is then placed on a clean cover-glass infected from the bacterial fluid, and covered with the slide in such a way that the drop is suspended in the middle of the cell. The sterilised broth is prepared in the same manner as nutrient gelatine, except that the addition of gelatine is omitted. For microscopical examination an oil-immersion lens with an Abbe's condenser and narrow diaphragm should be used. And where fungi are studied in this way, the edges of the drop should be observed with special care, since it is there that the morphological characteristics of the micro-organisms are best marked.

IV. THE TRANSMISSION OF PURE CULTIVATIONS TO ANIMALS.

This constitutes a very important part of bacteriological study. It may be conducted in many ways.

(*a.*) The animal is placed in a closed chamber, and the atmosphere is saturated with sterilised water containing the bacteria by means of a spray producer. Experiments of this kind are very valuable for the study of infectious diseases and of inhalation remedies.

(*b.*) The pure cultivation of a definite fungus is given to the animal with its food. In doing this the chief precaution necessary is to see that the food itself is innocuous. Koch's plan is to enclose the cultivation in a small starch capsule provided with a lid, and to place this on the back of the animal's tongue. Most of the bacteria that are devoid of spores appear to be destroyed by the action of the free acid in the stomach, and on this account it is advisable to neutralise the acid by the administration of alkalies, such as Koch used

in his cholera researches, or to perform laparotomy with strict antiseptic precautions, and to introduce the cultivation directly within the duodenum.

(*c.*) Cutaneous inoculation. The hair having been removed from some part of the body, as the ear, which the animal cannot easily reach with its tongue, a superficial wound is made there, and a portion of the cultivation is lodged within it.

(*d.*) In mice the inoculation is best made subcutaneously at the root of the tail. Or the purpose may be effected by infecting subcutaneously, or within one of the natural cavities, by means of Koch's modification of Pravaz's syringe. In this instrument a disc of cork is substituted for the india-rubber, which will not bear the great heat necessary to ensure sterilisation. A part of the cultivations suspended in water is sucked into the syringe, and the fluid injected beneath the animal's skin. A simple glass canula with a compressible india-rubber bulb will also serve.

V. SCHEME OF A BACTERIOLOGICAL INVESTIGATION.

1. The fluid to be examined is taken from the body with sterilised instruments and under the requisite precautions, and a drop is inspected microscopically, either with a powerful dry system or a homogeneous immersion objective; narrow diaphragms and an Abbe's condenser being also used. Dry preparations are made and stained. In doing this, solutions of the basic aniline dyes are used, or one of the other methods (p. 315), as those of Gram, Friedländer, &c., according to the character of the fungi supposed to be present.

2. Another drop of the fluid is added to fluid nutrient gelatine or agar-agar to obtain plate-cultivations.

The plate-cultivations are examined with the microscope after twelve to twenty-four hours, and it is seen whether they contain fungi which are identical with, or resemble in their mode of growth, others already known or under observation.

Should it appear that no pure cultivations have yet formed, other plates are made from those already prepared, until one is obtained in which a single fungus develops.

3. Drop-cultivations are made, and their growth observed directly. Moreover, these are nourished on various substances, such as potatoes, gluten, &c., and their behaviour to different nutrient fluids and under different conditions of temperature is studied.

4. Pure cultivations so obtained are transferred to different animals, and the morbid symptoms induced are observed. If such symptoms are analogous to those occurring in the human system in presence of

the same micro-organism, they may be assumed to be due in both cases to the agency of the latter.

When this is done, however, the resources of bacteriology are not yet exhausted. The biological characteristics of the fungi remain to be investigated, as to what sources of nitrogen, what sources of carbon, and what inorganic salts they require. It is only in this way that we can arrive at just conclusions concerning the nature of infectious diseases and attain to rational methods of anti-bacterial treatment.

APPENDIX.

I. Reaction of Blood (p. 2).—[The easiest method is to use a faintly-reddened *glazed* litmus paper. On it is placed a drop of blood, which is washed off with water after a few seconds. The spot where the blood rested is indicated by a blue patch on a red or violet ground (*Schäfer*).]

II. Method of Estimating the Specific Gravity of Blood (p. 5).—[In the method of *Landois*, instead of glycerine and water, solutions of sulphate of soda, varying in specific gravity from 1.050–1.070, are employed. A thin glass tube is drawn out in a gas-flame to form a capillary tube, which is bent at a right angle, and closed above with a small caoutchouc cap. A drop of blood is obtained from a finger, and by pressing lightly on the caoutchouc cap a quantity of the freshly-shed blood is drawn up into the capillary part of the tube. The tip of the fine capillary tube is at once immersed in one of the solutions of sodic sulphate and a drop of the blood expressed into the saline solution, and it is noted whether it sinks or floats. The operation is repeated with other solutions until one is found in which the blood neither sinks nor floats.]

III. The Blood-Plates or Platelets (p. 5).—[The blood-plates are exceedingly small (3–3.5 μ in diameter), round or oval disc-shaped bodies, homogeneous when quite fresh, but apt to assume a faintly granular appearance. They are non-nucleated. Being so pale and colourless, and rapidly disappearing in shed blood (apparently dissolving in the plasma), it is not to be wondered at that it was a long time before their existence was discovered. When they break up, their granular remains, consisting of some proteid matter, are apt to run together, and give rise to one form of what have been called the "granules" of the blood. As they can be seen within the living blood-vessels, there is no doubt as to their existence.

The blood-plates may be "fixed" by one of the following methods:—

(1.) Make a solution of .2 gramme of methyl-violet in 1000 cc. of

.75 per cent. salt solution. The methyl-violet dissolves somewhat slowly.

(2.) Mix one part of a 1 per cent. solution of osmic acid with two parts of .75 per cent. salt solution.

After carefully cleansing a part of the skin, place a drop of either solution (1 or 2) upon it, and with a clean needle puncture the skin *through* the drop of fluid, so that the blood flows directly into the drop of fluid. If the methyl-violet solution be used, the colourless corpuscles and also the blood-plates are stained of a bluish tinge, while with the osmic acid solution they are fixed and retain their shape permanently. In fact, such a preparation may be mounted in glycerine-jelly and preserved.]

IV. Bizzozero's Chromo-Cytometer (p. 12).—[According to Bizzozero,[1] a knowledge of the number of coloured blood-corpuscles is of less practical value than the quantity of hæmoglobin, and, as a matter of fact, the amount of the latter is not necessarily in direct proportion to the number of the former. For the purpose of estimating the *amount of hæmoglobin* Bizzozero has invented a small, practical, and handy instrument, which he calls a *chromo-cytometer*[2] (fig. 1).

By means of this instrument we can estimate the amount of hæmoglobin in the blood, and the instrument itself can be used either as a cytometer or as a chromometer. In both cases it depends upon a method of varying the thickness of a stratum of blood.

To use it as a cytometer, the blood is mixed with a definite volume (1 : 50) of an indifferent solution, *e.g.*, normal saline solution (0.75 gramme of sodic chloride in 100 cc. water), so that the corpuscles remain intact in the fluid. The quantity of hæmoglobin is estimated by the thickness of a layer of fluid through which one in a dark room can distinctly see the edges of a candle-flame placed at a distance of $1\frac{1}{2}$ metre from the instrument.

In using the instrument as a chromometer, the blood is mixed with a known volume of water, which dissolves out the hæmoglobin from the corpuscles. The amount of hæmoglobin is then calculated from the thickness of the layer of this mixture, which yields a colour exactly equal in intensity to that of a red-coloured glass supplied with the instrument.

The chief part of the instrument consists of two tubes (*ab*, *cd*), working one within the other, and closed at the same end by glass discs (figs. 1 and 2), while the other ends are open. The one tube can be completely screwed into the other, so that both glasses touch. Con-

[1] *Bizzozero*, Handbuch d. klin. Mikroskop., 2nd Germ. edit., transl. by Bernheimer. Erlangen, 1887.

[2] *Bizzozero*, Atti della Regia Acc. d. Sc. di Torino, vol. xiv., 1879.

nected with the outer tube is a small open reservoir (*r*), from which fluid can pass into the variable space between the two glass plates at the ends of the tubes. By rotating the inner tube, the space between the two glass plates can be increased or diminished, on the principle of Hermann's hæmatoscope, and the screw is so graduated as to indicate the distance between the two plates, *i.e.*, the thickness of the layer of fluid between them. Each complete turn of the screw = 0.5 mm., and the subdivisions on it are so marked—25 to one turn (index, fig. 1, *cd*)—that each subdivision of the index $= \frac{0.5}{25} = 0.02$ mm. When the inner tube is screwed home and touches the glass disc in the outer tube, the index stands at 0 on the scale. If the instrument is to be used merely as a cytometer, these parts suffice; but if it is to be used as a chromometer, the coloured glass must be used. The instrument is also provided with small glass thimbles with flat bottoms, containing 2 and 4 cc. respectively; a pipette graduated to hold $\frac{1}{2}$ and 1 cc., and another pipette for 10 and 20 cmm., the latter provided with an india-rubber tube, to enable the fluid to be sucked up readily; a bottle to hold the saline solution, and a glass stirrer.

Method of Using the Instrument as a Cytometer.—1. By means of the pipette place .5 cc. of normal saline solution in a glass thimble.

2. With a lancet or needle puncture the skin of the finger at the edge of the nail.

3. With the pipette suck up exactly 10 cmm. of blood, with the precautions already indicated at p. 9. Mix this blood with the .5 ccm. saline solution, and suck part of the latter several times into the capillary tube, so as to remove every trace of blood from the pipette. Mix the fluids thoroughly. Carefully cleanse the pipette with water.

4. Pour the mixture into the reservoir (*r*) of the instrument. Gradually rotate the inner tube, and as the two glass discs separate, the fluid passes into the space between them.

5. In a dark room light a stearin candle, place it at a distance of $1\frac{1}{2}$ metre, and, taking the instrument in the left hand, bring the open end of the tubes to the right eye. With the right hand rotate the inner tube to vary the thickness of the column of fluid, and so adjust it until the outlines of the upper three-fourths of the flame can be distinctly seen through the stratum of fluid. Vary the position of the inner screw so as to determine accurately when this occurs. Read off on the scale the thickness of the stratum of fluid.

Graduation of the Instrument as a Cytometer.—In this instrument the graduation is obtained from the thickness of the layer of blood itself, and the amount of hæmoglobin is calculated directly from the thickness of the layer of blood which is necessary to obtain a certain

optical effect, viz., through the layer of blood-corpuscles to see the outlines of a candle-flame placed at a certain distance.

From a number of investigations it appears that in healthy blood the outlines of the flame of a candle are distinctly seen through a layer of the mixture of blood $\frac{110}{100}$ mm. in thickness.

Let the number 110 correspond to 1, or, better still, 100 parts of hæmoglobin; then it is easy to calculate the relative value of the subdivisions of the scale on the tube of the instrument. Let g = the degree of the scale for normal blood; g', that for the blood being investigated; e, amount of hæmoglobin in the former; and e', the amount sought for in the latter.

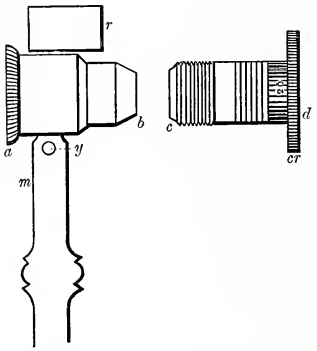


FIG. 1.—General view of the instrument. *ab* and *cd*. Two tubes, the one fits inside the other; *r*. Reservoir communicating with the space between *c* and *b* when *cd* is screwed into *ab*; *cr*. Milled head, and index-scale to the left of it; *y* for *ast* of fig. 3; *m*. Handle.

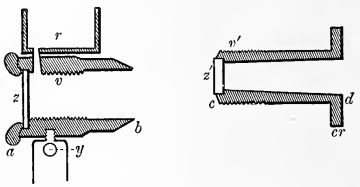


FIG. 2.—Showing how *cd* fits into *ab*. *z* and *z'*. Plates of glass closing the ends of *ab* and *cd*; other letters as in fig. 1.

Assuming that the product of the quantity of hæmoglobin and the thickness of the stratum of blood is constant, so that

$$e\,g = e'\,g'.$$

Then we have

$$e' = \frac{e\,g}{g'}.$$

Let us assume that the blood investigated gave the number 180; then, using the above data, we have:—

$$e' = \frac{100.110}{180} = \frac{11{,}000}{180} = 61.1.$$

The blood, therefore, contains 61.1 hæmoglobin. The following table

gives the proportion of hæmoglobin, the normal amount of hæmoglobin being taken as = 100 :—

Cytometer Scale.	Hæmoglobin.	Cytometer Scale.	Hæmoglobin.
110	100.0	170	64.7
120	91.6	180	61.1
130	84.6	190	57.9
140	78.5	200	55.0
150	73.3	210	52.4
160	68.7	220	50.0

If the instrument be used as a chromometer, the blood is mixed with a known volume of water, whereby the hæmoglobin is dissolved out of the red corpuscles and the fluid becomes transparent. The quantity of hæmoglobin is calculated from the thickness of the stratum of fluid required to correspond exactly to the colour-intensity of a coloured glass accompanying the instrument. The latter is coloured of a tint

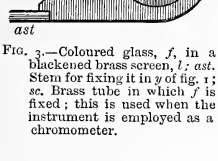


FIG. 3.—Coloured glass, *f*, in a blackened brass screen, *l*; *ast*. Stem for fixing it in *y* of fig. 1; *sc*. Brass tube in which *f* is fixed; this is used when the instrument is employed as a chromometer.

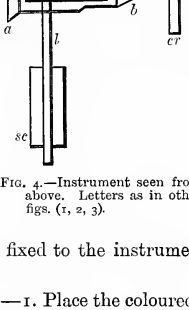


FIG. 4.—Instrument seen from above. Letters as in other figs. (1, 2, 3).

similar to a solution of hæmoglobin, and is fixed to the instrument by means of a suitable brass fixture (fig. 3, *f*).

Using the Instrument as a Chromometer.—1. Place the coloured glass with its brass frame in the instrument (fig. *ast* of fig. 3, in *y* of fig. 1).

2. With the necessary precautions (p. 9) mix 10 cmm. blood with .5 cc. distilled water, whereby in a few seconds a transparent solution of hæmoglobin is obtained.

3. Pour this solution into the reservoir (*r*), and rotate the inner tube so that the fluid passes between the two glasses. Direct the instrument towards a white light or the sky, not towards the sun, and compare the colour of the solution with the standard coloured glass, a procedure which is facilitated by placing a milky glass between the source of light and the layer of blood so as to obtain diffuse white light. When the two colours appear to have as near as possible the

same intensity, read off on the scale the thickness of the layer of blood, and from this by means of the accompanying table ascertain the corresponding amount of hæmoglobin.

This is done in the same way as for the cytometer, but the graduation is different, as in the one case we have to do with a candle-flame, and in the other with a coloured glass.

In very pronounced cases of anæmia, even with a layer of blood 6 mm. in thickness, the limits of the instrument, the intensity of the mixture of blood may be less than that of the coloured glass. In such a case, instead of 10 cmm. of blood, use 20 cmm.

Graduation of the Chromometer.—As the coloured glass has not absolutely the same intensity of colour in all chromometers, one must first of all estimate the colour-intensity of the glass itself. This is most easily done by ascertaining in a given specimen of blood what degree of the chromometer corresponds to the scale of the cytometer of the same blood.

Suppose that a specimen of blood by means of the cytometer gave 110, and by the chromometer 140; the number 110 of the cytometer = 100 hæmoglobin, so that the chromometer number 140 must also be = 100. With the aid of the formula (p. 332) a similar table can be constructed for the chromometer. Suppose the blood investigated = 280; then by the aid of the formula and the data from normal blood we have :—

$$e' = \frac{100.140}{280} = \frac{14,000}{280} = 50.$$

This blood, therefore, contains 50 parts of hæmoglobin.

Example.—Blood gives 130 with the cytometer and 190 with the chromometer; what is the initial number of the chromometer graduation corresponding to 100 parts of hæmoglobin?

If 130 (cytometer) corresponds to 190 (chromometer), then 110 cytometer (*i.e.*, graduation corresponding to 100 parts of hæmoglobin) corresponds to x chromometer graduation :

$$130 : 190 = 110 : x \therefore x = \frac{190.110}{130} = \frac{20,900}{130} = 160.7.$$

Blood containing 100 parts hæmoglobin will correspond to 160 of the chromometer scale, and beginning with this number as a basis, with the aid of our formula it is easy to construct a table showing the relation.

Whilst the value of the cytometer scale remains the same for every instrument, the chromometer scale varies with each instrument, as the colour-intensity of the glass is not necessarily the same in all. But it is easy to construct a scale for each instrument by investigating

a specimen of blood and comparing it with the cytometer graduation as indicated in the foregoing paragraph.

Precautions to be Observed in Using the Instrument.—In using the instrument certain precautions must be observed. The exact quantity of the several fluids must be carefully measured; evaporation must be prevented by covering the blood-mixture. Further, do not look at the fluid too long at a time, as the eye becomes rapidly fatigued.

In cases of leukæmia, where there is a large number of white corpuscles rendering the mixed fluid opaque, the corpuscles may be made to disappear by adding a drop of very dilute caustic potash. If the opacity does not disappear by the addition of this substance, then the opacity is due to the presence of fatty granules in the blood, so that by this means we can distinguish lipæmia from leukæmia.

Further, the operation must be carried out not too slowly, as the saline solution only retards the coagulation of the blood, and does not arrest it.

Bizzozero claims that when the instrument is used as a cytometer the mean error is not greater than 0.3 per cent.*]

V. Amount of Hæmoglobin (p. 14).—According to Preyer, the quantity of hæmoglobin is 13.77 per cent. in man and 12.59 per cent. in woman. Similar results are obtained by calculating the amount of hæmoglobin from (1) the amount of iron, or (2) the colour method, or (3) from the spectrum.[1]

VI. "Granules" in Colourless Blood-Corpuscles (p. 22).—[Ehrlich and his pupils[2] have shown that cells, or certain "granules" in cells, exercise a selective power in relation to aniline pigments; thus, some granules are coloured by one dye, and others by a different dye. According to Ehrlich, there are three kinds of granules or granulations in relation to aniline pigments.[3] (1.) "**Eosinophile granules**," or those granules which are stained specially by *acid* pigments, *e.g.*, orange, ponceau, acid fuchsin, aurantia, and specially by eosin. (2.) "**Basophile granules**," which are stained by *basic* aniline dyes, *e.g.*, dahlia, gentian-violet, methyl-violet, fuchsin, methyl-green, acetate of rosanilin, vesuvin. (3.) "**Neutrophile granules**," those that are specially stained by neutral dyes, *i.e.*, those composed of a coloured base and an acid, *e.g.*, methyl-blue and acid fuchsin. The last variety

* The instrument may be obtained from F. Koristka, Via Circo, 14, Milan, and costs thirty-five lires.

[1] *Preyer*, Die Blutkrystalle, p. 126. Jena, 1871.

[2] *Ehrlich*, Verhandl. d. phys. Gesell. zu Berlin, No. 20, 1878–79; Charité-Annalen, p. 107, 1884.—*Bizzozero*, Handb. d. klin. Mikros., Erlangen, 1887.

[3] *Ehrlich*, Zeits. f. klin. Med., i., p. 553; Charité-Annalen, 1884, p. 107.—*Eugen Westphal*, Ueber Mastzellen, Inaug.-Diss., Berlin, 1880, pp. 18–19.—*Nordmann*, Beiträge z. Kenntniss v. namentlich z. Färb. d. Mastzellen, Inaug.-Diss., Berlin, 1884.

consists of very fine granules, which require a good immersion lens to see them.

The white corpuscles containing neutrophile granules are most abundant in human blood; those with basophile granules do not seem to exist in normal blood; while the eosinophile variety occurs but sparingly. The basophile variety is found but seldom in diseased conditions. It occurs in the white corpuscles of the blood of the newt and in the so-called "Mastzellen" in connective tissue.

For the *eosinophile* granules an eosin-glycerine solution is the best.

For the *basophile*, use a solution of methyl-green or an acid solution of dahlia.

Water	100 ccm.
Saturated alcoholic solution of dahlia . .	50 ,,
Glacial acetic acid	10 ,,

For the *neutrophile* granules Ehrlich recommends the following mixture:—To 5 parts of a saturated solution of acid fuchsin add 1 part of a concentrated solution of methyl-blue—stir all the time—and add 5 parts of distilled water. After several days filter the mixture.[1]

Dr. S. Delépine divides the aniline dyes into two classes. (1.) Acid fuchsin may be taken as a type of the first; (2.) basic fuchsin as a type of the second. The first class stains deeply the most differentiated parts of the cells; the second, the least differentiated parts of the cells. In a drop of blood, the red corpuscles are most deeply stained by acid fuchsin, the leucocytes but faintly stained, while the nuclei are not stained at all. If basic fuchsin be used, the nuclei are stained most deeply, the body of the leucocytes to a less extent, and the red corpuscles least of all.[2]]

VII. Stokes's Fluid (p. 41).—[Make a watery solution of ferrous sulphate; to it add liquor ammoniæ after the previous addition of sufficient tartaric acid to prevent precipitation. It is usual to add about three parts by weight of tartaric acid to two parts of the iron salt. It should be made fresh as required.]

VIII. Claude Bernard's Method of Estimating Grape-Sugar in Blood (p. 48).—[Place 20 grammes of crystallised sodic sulphate in each of six porcelain capsules, and to each add exactly 20 grammes of the blood to be investigated. Mix the blood and salt together. Boil them until the froth above the clot becomes white, and the clot itself does not present any red specks. Weigh again, and make up the loss by evaporation by the addition of water. The whole is then

[1] *Bizzozero*, Handb. d. klin. Microscop., Erlangen, p. 43, 1887.

[2] *Lauder Brunton*, Brit. Med. Journal, p. 1276, 1889.

placed in a small press, and the fluid part expressed, collected in a capsule, and afterwards filtered. The filtrate is placed in a burette.

In a flask place 1 cc. of Fehling's solution, and to it add a few small pieces of caustic potash and 20 cc. of distilled water. Boil this fluid, and from the burette allow the clear filtrate of the blood to drop into the boiling dilute Fehling's solution until the latter loses every trace of its blue colour. As in all sugar estimations, the process must be repeated several times to get accurate results. Hence the reason why several capsules are prepared.

Read off the number of cc. used of the filtrate in the burette, *e.g.*, $= n$ cc. The formula

$$S = \frac{8}{n} =$$

in grammes the weight of sugar per kilogramme of blood.

In Seegen's method, which may be taken as the type of the newer methods, the proteids are precipitated by ferric acetate. The blood is diluted with 8–10 times its volume of water, acidulated with acetic acid, and heated. When the precipitation of proteids commences, render the mixture strongly acid by the addition of acetate of soda and perchloride of iron; then add sufficient sodic carbonate until the mixture is faintly acid, and boil. Allow it to cool, and filter it through a fine cloth filter, free from starch. The filtrate ought to be clear. The residue on the filter is washed several times with water, and the remaining fluid in it expressed by means of a small hand-press. The expressed fluid is then mixed with the clear filtrate if the mixture has a slightly reddish tint from the admixture of a small quantity of blood-pigment. Add a drop or two of perchloride of iron to precipitate the last traces of the proteids. Filter again. The sugar in the filtrate is estimated in the usual way by means of Fehling's solution.[1]]

IX. Griess' Papers (p. 56).—[As a test for nitrites or ozone in saliva; these can be obtained from Schuchard, Chemische Fabrik, in Görlitz.]

X. Staining of Tubercle Bacilli in Sputum (p. 78).—[The basic aniline dyes stain these bodies, but some sort of mordant is required to fix the dye in the organism, or at least render it less liable to be removed by the action of decolorising reagents. Amongst these mordant-like acting substances are carbolic acid, aniline oil, ammonia, borax, &c.

Gibbes' Method.—Take

(I.)	Rosaniline hydrochloride . . .	3 grammes.
	Methyl-blue	1 gramme.

[1] *Beaunis*, Nouveaux Eléments de Physiologie, 3rd edit., p. 138, 1888.—*Seegen's* Papers in Pflüger's Archiv, xxxiv. 1884, xxxvii. 1885, xxxix. 1886.

Mix these thoroughly by rubbing them together in a mortar.

(II.)	Aniline oil	5 cc.
	Rectified spirit	20 ,,

Dissolve, gradually add II. to I., stirring vigorously all the while, and when I. is completely dissolved, add 20 cc. distilled water, and keep the mixture in a stoppered bottle.

A thin layer of the sputum is obtained on a cover-glass in the usual way. A little of the mixture is warmed in a watch-glass, and the cover-slip—sputum surface downwards—is floated on the warm staining fluid for four or five minutes. It is removed and washed with spirit by means of a wash-bottle, until the latter ceases to remove any of the dye. The preparation is allowed to dry, and mounted in xylol balsam.[1]

Gabbett's Method.[2]—Two solutions are required.

A. *Magenta Solution.*

Carbolic acid (5 per cent.) . . .	100 cc.
Magenta	1 gramme.
Spirit	10 cc.

Mix and preserve in a stoppered bottle.

B. *Methylene-Blue Solution.*

Sulphuric acid (25 per cent.) . . .	100 cc.
Methylene-blue	2 grammes.

The prepared covers are floated on a little of the magenta solution in a watch-glass. The latter is heated just until the vapour can be seen rising from the surface. The cover-glasses are then removed, washed, and placed for one to two minutes in the methylene-blue solution; after removal they are again thoroughly washed, dried, and mounted in xylol balsam. The whole process can be accomplished in a very few minutes.]

XI. Triple Phosphate (p. 83).—[This assumes various crystalline forms (see *Urine*), one of the varieties being called the knife-rest form. Their formation is due to the decomposition of proteids, whereby ammonia is set free. The proteids yield the necessary ammonia to combine with phosphate of magnesia to form the phosphate of magnesia and ammonia, ammonio-magnesic phosphate or triple phosphate.]

XII. Test for Hydrochloric Acid (p. 101).—[Dreschfeld[3] has

[1] *Gibbes*, Pract. Histol. and Path., London, 1885.

[2] *Gabbett*, Lancet, April 1887; Korrespondenzb. f. schweizer Aerzte, 1887; and a paper by *Ernst* in Centralb. f. klin. Med., 1888, No. 32, p. 573.

[3] *Dreschfeld*, Clinical Diagnosis of Cancer of the Stomach, Medical Chronicle, May and June, 1886.

employed this test for the presence of hydrochloric acid. After using the methyl-violet test, he uses Uffelmann's test as follows. The test solution consists of 0.5 ccm. of claret (unadulterated), 3 ccm. of 90 per cent. alcohol, and 3 ccm. of ether; which solution is almost colourless, and is rendered a rose colour by the presence of a minute quantity of hydrochloric acid. This test is said by Dreschfeld not to be interfered with by the presence of peptone or albumin. Lactic acid gives a similar reaction only when occurring in a more concentrated form. The mixture does not keep long, and has to be freshly prepared.]

XIII. Proteids Formed during Gastric Digestion (p. 104).—[When fibrin or other proteid is acted upon artificially by pepsin and hydrochloric acid in the presence of water and at a suitable temperature, 40° C., the proteid passes through several stages before it is converted into the final product or peptone. In reality, the proteid molecule seems to be split up, and the *albumoses* are the first bodies formed.

Kühne, to whose investigations our knowledge of this subject is principally due, in his earlier communications described *propeptone* or *hemi-albumose* as the first intermediate substance so formed. By his later researches he has shown that hemi-albumose is in reality a complex body made up of three albumoses, called *proto-albumose*, *hetero-albumose*, and *deutero-albumose*, which differ from each other slightly, and can be distinguished in a gastric digest by certain chemical tests. So far, these three bodies have not been isolated from the actual product of gastric digestion taken from the stomach of a living digesting animal.]

XIV. Dipteral Larvæ Passed Alive from the Bowel of a Man (p. 155).—[For an account of a remarkable case of this nature see *British Medical Journal*, June 8, 1889, as recorded by Dr. James Finlayson. The larvæ were passed alive in swarms from the bowel of a man. The insect itself was the Anthomyia canicularis or scalaris. An account is also given of the literature of the subject.]

XV. Gases in the Stomach and Intestine (p. 164).

[*Gases in the Stomach.—Vol. per cent.* (*Planer*).

	Human Subject after Vegetable Diet.		Dog.	
	I.	II.	I. After Animal Diet.	II. After Legumes.
CO_2	20.79	33.83	25.2	32.9
H	6.71	27.58	...	...
N	72.50	38.22	68.7	66.3
O	...	0 37	6.1	0.8

Gases Discharged per Rectum in Man (Runge).

Food.	Milk.		Flesh.		Legumes.		
	I.	II.	I.	II.	I.	II.	III.
CO_2	16.8	9.9	13.6	12.4	34.0	38.4	21.0
H	43.3	54.2	3.0	2.1	2.3	1.5	4.0
CH_4	0.9		37.4	27.5	44.5	49.3	55.9
N	38.3	36.7	45.9	57.8	19.1	10.6	18.9

Intestinal Gases.—Ewald gives the intestinal gases as CO_2, H, N, H_2S, and CH_4. The marsh-gas, or CH_4, is due to a special fermentation, and seems to be formed chiefly from the cellulose taken in with the vegetable food. The gases vary very much according to the nature of the food. In gases obtained by puncture from the intestine in a case of typhus with meteorism, Ewald found 8.2 per cent. of CO_2 and the remainder N. Easily fermentable vegetables, such as cabbages, frequently lead to the formation of a large quantity of gases.]

XVI. Colour-Scale for Urine Testing (p. 174).—[The colour-scale for urine adopted by Vogel is: (1.) Pale-yellow; (2.) Bright-yellow; (3.) Yellow; (4.) Reddish-yellow; (5.) Yellowish-red; (6.) Red; (7.) Brownish-red; (8.) Reddish-brown; and (9.) Brownish-black.

In connection with the colour of normal and pathological urines, the following table from Krukenberg[1] will be found useful :—

[1] *Krukenberg*, Grundriss d. med.-chem. Analyse, 1884; and *MacMunn*, Clinical Chem. of Urine, 1889.

Colour of Urine.	Cause of Colouration.	Pathological Condition.
Pale-yellow to colourless.	Diminution of normal pigments.	Anæmia, chlorosis, diabetes, and after nervous attacks.
Dark-yellow to brown-red; easily depositing a sediment.	Increase of normal, or occurrence of pathological pigments.	Acute febrile diseases.
Yellowish and milky.	Fatty drops floating in the urine.	Chyluria.
	Suspended pus corpuscles.	Pyelitis or other purulent discharges.
Green or yellowish-green.	Bile colouring-matter.	Jaundice.
Greenish-yellow, later greenish-brown, or approaching black.	Decomposed hæmoglobin, and various chromogens. Carbolic urine is similar.	Hæmorrhages into the kidneys, long-continued intermittent fever, also melanotic cancer.
Red or reddish.	Unchanged hæmoglobin.	Hæmorrhages or hæmoglobinuria (?).
	Pigments which are taken in with the food, *e.g.*, madder, bilberries, logwood, fuchsin.	
Brown.	Bile colouring-matter and methæmoglobin.	
Brown-yellow to red-brown, becoming blood-red on adding alkalies.	Substances which are introduced into the organism with senna and rhubarb, &c.	

According to MacMunn,[1] the principal normal colouring-matter in urine is **normal urobilin,** which is a nitrogenous, amorphous, yellowish-brown pigment, soluble in alcohol, chloroform, acids, and partially in ether and benzol. It appears to exist in urine partly as a chromogen, and seems to be identical with choletelin. MacMunn regards it as an oxidation-product of effete hæmatin and bile-pigments, not a reduction-product, so that, according to him, it is not identical with hydrobilirubin.]

XVII. Reaction of Urine (p. 174).—[That the normal acid reaction of urine is not due to a free acid is shown by the fact that

[1] *MacMunn*, Proc. Roy. Soc., No. 208, 1880; Jour. of Phys., x., Nos. 1 and 2, and Clin. Chem., p. 104, 1889.

it gives no precipitate with sodium hyposulphite. Brücke has recently studied its relations to congo-red. Urine does not change the colour of papers or threads saturated with a watery solution of congo-red, while water containing 1 in 50,000 of free hippuric acid makes such papers or threads of a violet tint.]

XVIII. Albuminuria (p. 206).—[As to the frequency of occurrence of albuminuria in healthy people, great differences of opinion exist. It may be well to note here the opinion of Professor Grainger Stewart. He is of opinion that "albumen, if present at all in normal urine, is in such extremely minute amount as to be barely discernible, or not discoverable at all with the most delicate tests, even after considerable concentration; and that at least in some of the cases in which minute traces occur it is accounted for, not by transudation with the urine, but by the after addition of epithelial and other cellular elements from the urinary passages." [1]

Professor Stewart has made a large number of observations on the incidence of albuminuria in presumably healthy persons, including soldiers, male adults in civil employment, and the healthy inmates of a large poorhouse. Of 505 presumably healthy persons whose urine was examined—the urine being collected in the forenoon or at noon—32.8 per cent. showed evidence of the presence of albumen. But there was a marked difference between soldiers and men of corresponding age following civil occupations. Amongst the soldiers and recruits examined, 37.56 per cent. had albuminuria, and of the civil population 10 per cent.

There is further a marked contrast between the urine of children and that of people about or above sixty years of age, yet all the latter presumably healthy. In children the percentage showing albumen was 17; in people about or above sixty, 62.]

XIX. Proteids in Urine (p. 220).—[Dr. Sydney Martin [2] in this country has devoted attention to the proteids in urine. Saturation of the urine (this requires some time, the urine must be thoroughly shaken up for a considerable time with a large excess of the salt) with powdered *neutral*-ammonium sulphate precipitates the albumens, globulins, and albumoses; but the peptones, as is well known, remain in solution. The precipitate obtained by the ammonium salt rises to the surface of the liquid, and can be separated by filtration. Wash the precipitate with a cold saturated watery solution of neutral ammonium-sulphate. The precipitate can then be dissolved in distilled water. From this concentrated solution the globulins can be precipitated by

[1] *Grainger Stewart*, On Albuminuria, p. 19, 1888.
[2] *S. Martin*, Brit. Med. Jour., April 1888, pp. 842–844.

saturation with magnesium-sulphate crystals, while the filtrate contains the serum-albumin.]

XX. Detection of Serum-Globulin in Urine (p. 222).—[If serum-albumin and serum-globulin are together present in the urine, the latter may be precipitated by saturating the urine with crystals of magnesium-sulphate, or a saturated solution of the latter fluid may be poured into a test-tube containing the urine. If serum-globulin in any quantity be present, a milky ring forms where the heavy magnesium-sulphate solution comes into contact with the lighter urine which rests on it.

Upon the fact that globulins are insoluble in water and soluble in weak saline solutions, Sir William Roberts based a simple method for the detection of serum-globulin in urine. Fill a comparatively tall glass vessel with distilled water, and allow the urine, drop by drop, to fall into it. It is well to have a black background, when each drop of urine, as it falls into and mingles with the distilled water, shows a milky trace or milky way, the whitish deposit being more pronounced the richer the urine is in globulin. After a sufficient amount of urine is added—provided the urine contains serum-globulin—a very considerable deposit of the latter may be obtained. The deposit is readily soluble, like all globulins, in a weak acid, *e.g.*, acetic, and in a few drops of a solution of sodic, magnesic, or ammonic sulphate.]

XXI. Estimation of Grape-Sugar in Urine by Fermentation (p. 232).—[When beer yeast is mixed with saccharine urine, the grape-sugar present in the latter is split up into, amongst other bodies, ethylic alcohol and carbon dioxide, and it is found that about 95 per cent. of the sugar is split up into alcohol and CO_2—the best temperature to effect this being about 35° C. The fluid gives off CO_2, and becomes lighter in colour, while its specific gravity diminishes.

Sir William Roberts states that urine containing less than 0.5 per cent. does not show signs of fermentation when treated with brewer's yeast, so that it is not a very delicate test.[1]]

XXII. The Urine in Febrile States (p. 273).—[MacMunn has described a body, **febrile** or **pathological urobilin**, as occurring in urine. While obtained in the same way as normal urobilin, its spectrum differs from that of the latter body. It seems to represent a less oxidised state than does normal urobilin. This body appears to be so closely allied to, as almost to be indistinguishable from, the substance known as "stercobilin"—the colouring-matter of the fæces. MacMunn regards pathological urobilin as identical with stercobilin. If this be so, then

[1] *Roberts*, Pract. Treat. on Urinary and Renal Diseases, 4th edit.

the presence of pathological urobilin is, to a certain extent, an indication of the absorption of fæcal matters from the intestine.]

XXIII. Chylous Ascites (p. 301).—[An interesting case of chylous ascites is recorded by W. Whitla.[1] The patient, a boy aged 13, was tapped several times; on the first occasion, twelve pints of a milky fluid were removed from the abdominal cavity. The fluid under the microscope presented the characters of chyle. When tapped on another occasion, twenty-two pints of fluid, with all the appearances of milk, were drawn off. Part of this fluid was analysed by Matthew Hay, with the following result:—

Water	94.085 per cent.
Solids	5.915 ,,
Inorganic solids	0.995 per cent.
Organic	4.920 ,,
Albumin	2.878 per cent.
Fat	1.030 ,,
Sugar	0.210 ,,
Other organic matter	0.802 ,,

On *post-mortem* examination, the receptaculum chyli was found to be perforated, so that the chyle coming from the intestine was poured into the peritoneal cavity. Altogether, 117½ pints, or nearly 15 gallons, of chyle were removed from the body during life. During a period of sixty-eight days 2110 oz. were removed, or rather more than 31 oz. per day.

In this connection it is extremely interesting to recall the fact—first shown by Ludwig—that ligature of the thoracic duct in the dog is ultimately followed by rupture of the receptaculum chyli, so that the lower limbs become œdematous while chyle is discharged into the peritoneal cavity. This shows what powerful forces are concerned in propelling the chyle from the small intestine into and through the lacteals and thoracic duct.]

XXIV. Abbe's Condenser and Apochromatic Lenses (p. 314).—[For bacteriological work, it is convenient to have a microscope with a stand to which an Abbe's condenser can be fixed under the stage. Fig. 5 shows a convenient form made by Reichert of Vienna. Other makers furnish somewhat similar stands. Abbe's condenser is placed under the stage, and the tube of the microscope is provided with a "revolver" or "nose-piece," to which lenses of different magnifying powers are fixed.

[1] Brit. Med. Journal, 1885, p. 1089.

In an Abbe's condenser (fig. 6) the illuminating apparatus is a condenser system of very short focus (the section of the lenses is shown

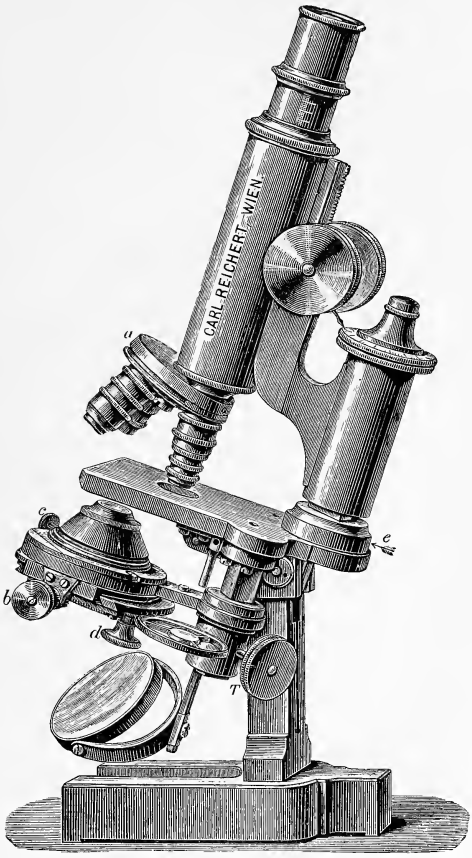


Fig. 5.—Reichert's Stand No. II.*b*., with draw-tube, rack-and-pinion fine adjustment, and Abbe's condenser.

in fig. 7), which collects the light reflected by the mirror (*Sp*)—the plane side of the mirror being used—into a cone of rays of very large

aperture, and projects it on the object. For ordinary work the cone of light is reduced by means of diaphragms, the most convenient form

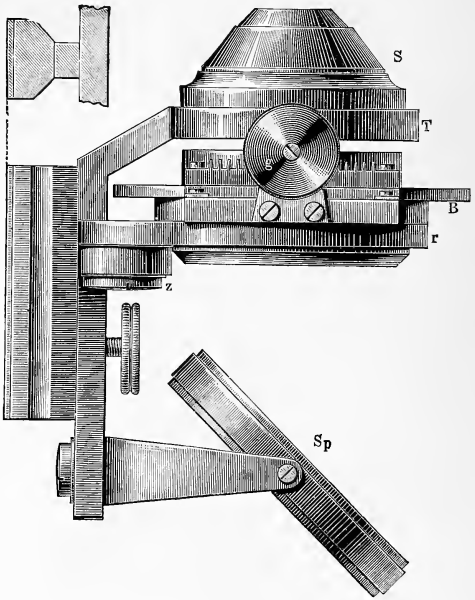


FIG. 6.—Abbe's Condenser, as made by Zeiss. Natural size, side view. *Sp.* mirror.

being that known as the "iris diaphragm," which can be adjusted to any size (fig. 7, *d*). Oblique illumination can be obtained by placing the diaphragm excentrically, which is done by means of the rack-and-pinion movement (*e*).

When very exact definition is required, apochromatic lenses are employed. They are expensive, but the objectives are so constructed as to secure the union of three different colours of the spectrum in one point of the axis. The images projected by them are nearly equally sharp with all the colours of the spectrum. As there is very great concentration of light by these objectives, they permit of the use of very high eye-pieces, thus giving high magnifying power with relatively long focal length. A series of *compensating* oculars are used with these lenses. The eye-pieces of extremely low power are called

"searchers," while the ordinary or working eye-pieces, beginning with a magnifying power of 4, are classified as 4, 8, 12, 18, and 27. With

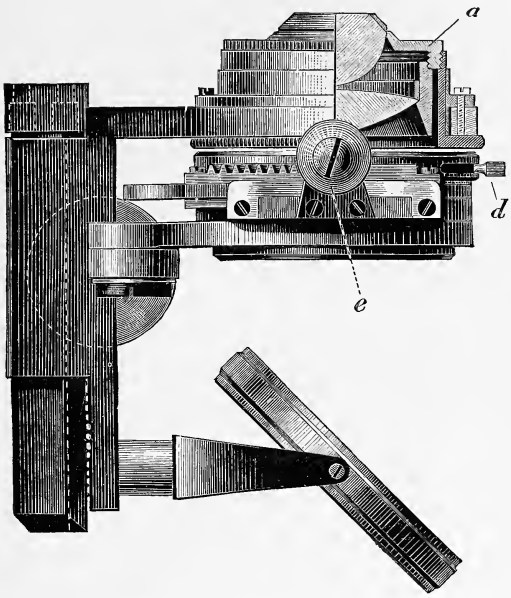


FIG. 7.—Abbe's Condenser as above, showing the collecting system of lenses.
d. Iris diaphragm.

these eye-pieces great magnifying power is obtained, partly by the lens, but also by the ocular used.

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CHAPTER VI.

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CHAPTER VII.

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CHAPTER X.

BACTERIOLOGICAL RESEARCH.

[1] The following list includes the most important works on Bacteriology, with special reference to those which describe the methods of examination and the morphology of Bacteria. We direct attention, as a basis of study, above all, to the already-mentioned works of *R. Koch* and his school.—*Crookshank*, Chap. iv. (33)—*Flügge*, Chap. i. (62).—*Cornil* and *Babes*, Les Bacteries. Paris, 1885.—*C. Fränkel*, Grundriss der Bacterienkunde. Berlin, 1887.—*A. Johne*, Ueber die Koch'schen Reinculturen und die Cholerabacillen. Leipzig, 1885.—*W. Zopf*, Die Spaltpilze, 3rd edit. Berlin, 1885.—*C. Friedländer* and *Eberth*, Mikroskopische Technik, 4th ed., 1890.—*Siebenmann*, Die Fadenpilze. Wiesbaden, 1883.—*A. de Bary*, Vergleichende Morphologie und Biologie der Pilze, Leipzig, 1884; and *A. de Bary*, Vorlesungen über

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INDEX.

ABBE'S condenser, 312, 346
Abbe-Zeiss counting cell, 8
Abscess, pulmonary, 82, 90
—— pus from, 291
Absorption bands, 38, 39, 40, 41, 241
Absorption, rate of, in stomach, 106
Accidental albuminuria, 209
Acetic acid, 103, 161, 251
—— acid in fæces, 161
—— acid in gastric juice, 102
—— acid in sputum, 84
—— acid in urine, 251
—— acid reaction in gastric juice, 98
—— acid tests for, 251
—— acid and ferrocyanide of potassium test for albumin, 210
Acetonæmia, 51
Acetone, auto-intoxication with, 249
—— in blood, 51
—— detection of, 299
—— in pus, 299
—— in urine, 249
—— tests for, 250
Acetonuria, 249
Acetphenetidin, 287
Acetylparaamidophenol, 287
Acholic stools, 169
Acid, acetic, 84, 103, 161, 251
—— α-crotonic, 280
—— æther-sulphuric, 241
—— albumin, 210
—— benzoic, 225
—— butyric, 103, 161
—— capric, 162
—— caproic, 84, 162
—— carbolic, tests for, 122
—— carbolic, estimation of, 246
—— chrysophanic, 288
—— diacetic, 250
—— diazobenzol-sulphonic, 273
—— formic, 49, 98, 161, 251
—— hippuric, 198
—— hydrochloric, 96
—— hydrocyanic, 123
—— hydroparacumaric, 244
—— hyposulphurous, 271
—— indoxyl-sulphuric, 242
—— isobutyric, 161
—— lactic, 49, 103, 160, 280
—— metatungstic, 120
Acid, nitric, 112
—— oleic, 162
—— oxalic, 112, 252
—— oxyamygdalic, 279
—— palmitic, 162
—— paraamido-phenol-sulphuric, 285
—— parakresol-æther-sulphuric, 244
—— paroxy-phenyl-acetic, 244
—— paroxy-phenyl-propionic, 244
—— phenol-æther-sulphuric, 244
—— phosphates, 268
—— phosphomolybdic, 120
—— phosphotungstic, 219
—— phosphoric, 269
—— picric, 212
—— pigments, 335
—— pimaric, 210
—— pinic, 210
—— propionic, 161, 251
—— sarcolactic, 49, 281
—— skatoxyl-sulphuric, 244
—— stearic, 162
—— succinic, 302
—— sulphanilic, 273
—— sulphuric, 111, 241, 268
—— uric, 47, 195, 254
—— valerianic, 162
Acid-forming fungi, 321
Acidity of gastric juice, 96; action on fungi, 325
—— of urine, 174
Acids in fæces, 160
—— organic, in blood, 49
—— organic, in fæces, 160, 162
—— organic, in gastric juice, 103
—— organic, in sputum, 84
—— organic, in urine, 251, 280
—— poisoning with, 111, 281
—— volatile fatty, 49, 84, 103, 160, 251, 280
Actinomyces, 293
—— in blood, 55
—— in buccal secretion, 59
—— in pus, 293
—— in sputum, 79
—— in urine, 192
Acute bronchitis, 85
—— enteritis, 165
—— gastritis, 108
—— nephritis, 274

Acute yellow atrophy, 278
Addison's disease, urine in, 240
Aërometer, 57
Æther-sulphuric acids, 241, 284
—— tests for, 244
—— estimation of, 245
Æthylinimin, 81, 306
Agar-agar, cultivations in, 135
—— nutrient, 319
Albumin, estimation of, in urine, 213
—— in cysts, 303
—— in fæces, 158
—— in gastric juice, 104
—— in pus, 298
—— in sputum, 84
—— in transudations, 301
—— in urine, 206
—— tests for, 209; *Fürbringer's*, 211; *Hindenlang's*, 211; *Heller's*, 211; *Heynsius'*, 211; *Johnson's*, 212
Albuminate, 210
Albuminimeter, 216
Albuminous putrefaction, 242
Albuminuria, 207, 342
—— accidental, 209
—— *Bright* on, 206
—— causation of, 207
—— experimental, 206
—— febrile, 208
—— hæmatogenic, 208, 280
—— in boys, 206
—— intermittent, 208
—— of anæmia, 208
—— pathological, 207
—— physiological, 207
—— renal, 207
—— *Stewart* on, 342
Albumose in serum, 308
Albumosuria, 207, 220
Alcapton, 247
Alcohol, detection of, 121
Alcoholic poisoning, 121, 283
Alimentary canal, diseases of, fæces in, 165
—— canal, diseases of, urine in, 278
—— canal, diseases of, vomit in, 108
Alkalies, poisoning with, 112, 281
Alkaline stools, 125
—— urine, 174
Alkalinity of the blood, 2, 4
—— of the blood, changes of, in disease, 4
—— of the blood, estimation of, 2
—— of the blood, *Hayeraft* and *Williamson's* test for, 4
—— of the blood, *Liebreich's* test for, 2
—— of the blood, *Zuntz'* test for, 2
—— of the urine, 174
Alkaloids, animal, 118, 262
—— poisoning with, 117, 283
—— *Stas-Otto* tests for, 117
Allantoin, 46, 225, 302
Almén's fluid, 228
—— test, 282
Alt's test for mercury, 282
Alum-carmine, 61
Alveolar epithelium, 67, 89
Amido-acids, 256
Ammonia, urate of, in sediment, 203
—— in gastric juice, 105
—— in vomit, 112
Ammoniacal fermentation, 174
—— silver solution, 254
Ammoniæmia, 51, 118
Ammonia-salts, in gastric juice, 105
—— estimation of, 105
—— in urine, 271
—— in vomit, 112
—— poisoning with, 112
Ammonio-magnesic phosphate, 83, 157, 197, 202, 298, 338
Amœba coli, 140
Amœbæ in urine, 193
Amorphous hæmatoidin, 26
—— sediment of urine, 201, 204
Amphoteric urine, 174
Amyloid concretions, 306
—— kidney, 179, 275
Anæmia, albuminuria in, 206
—— blood in, 25
—— pernicious, blood in, 25
—— urine in, 173, 280
α-naphthol, 230
Anchylostoma duodenale, 111, 150
Anchylostomiasis, 151
Angina, croupous, 61
—— diphtheritic, 61
—— Ludovici, 292
Anguillula intestinalis, 154
—— stercoralis, 154
Aniline, 75, 122
—— -brown, 27, 28
—— dyes, 28, 315
—— oil, 75
—— poisoning with, 122
—— poisoning, urine in, 285
—— tests for, 123
—— tests for hydrochloric acid, 97
—— -water, gentian-violet, 75
Animal alkaloids, 118, 262
—— alkaloids, tests for, 119, 262
—— gum, 237
—— parasites of blood, 35
Annelides, 35, 148
Anthelmintics, 150, 151
Anthomyia canicularis, 339
Anthomyiæ in fæces, 155, 339
Anthracosis of lung, 92
Anthrax, bacillus of, 29, 296
—— bacillus of, detection of, 30, 297
—— bacillus of, in blood, 29
—— bacillus of, in pus, 296
Antifebrin in urine, 287
—— test for, 122
Antipyrin in urine, 173, 286
Antisepsis of urine, 175
Anuria, 171
Apochromatic lens, 314, 346
Areolar tissue in stools, 127
—— tissue in vomit, 107

Aromatic oxy-acids, 244
Arsenic, detection of, 115
—— poisoning with, 115, 168, 282
Arterio-sclerosis, 284
Arthritis, 47, 256, 269
Ascarides in fæces, 148
—— in nose, 64
—— in sputum, 80
—— in urine, 194
Ascaris lumbricoides, 147
—— mystax, 148
Ascites, chylous, 343
Asiatic cholera, bacillus of, 133
—— fæces in, 168
Aspergillus fumigatus, 74
Asphyxia, 224
Asthma, 85
—— bronchial, 69
Atrophic cirrhosis, urine in, 279
Atrophy, gastric, 109
Atropin, detection of, 118
—— in urine, 283
—— poisoning with, 118, 283
Aurantia, 22
Auto-intoxication with acetone, 249
—— with diacetic acid, 250
—— with sulphuretted hydrogen, 272
Automatic pipette, 3, 13
Autotoxicosis, 264
Azoospermia, 306

Bacilli in blood, 26–35
—— in buccal secretion, 53, 58, 61
—— in exudations, 291
—— in fæces, 131
—— in gastric juice, 108
—— in milk, 309
—— in nasal secretion, 64
—— in urine, 190
—— in vagina, 310
Bacilluria, *see* Bacteriuria
Bacillus, crassus sputigenus, 54
—— cyanogenus, 309
—— *Lustgarten's*, 292
—— malariæ, 33
—— of anthrax, 29, 296
—— of Asiatic cholera, 133
—— of cholera infantum, 125
—— of cholera nostras, 137
—— of decaying teeth, 54
—— of *Finkler* and *Prior*, 137
—— of glanders, 32, 191, 296
—— of hay, 139
—— of hen-cholera, 315
—— of hydrophobia, 34
—— of lactic-acid, 165
—— of leprosy, 297
—— of septicæmia of the mouse, 132
—— of smegma, 292
—— of syphilis, 292
—— of tetanus, 34
—— of tubercle, 32, 75, 139, 292, 305, 337
—— of typhoid, 33, 138, 191
Bacillus of whooping-cough, 79
—— salivarius septicus, 54
—— septicus sputigenus, 54
—— subtilis, 131
Bacteria, *see* Fission-fungi
Bacterial colonies or casts, 180
—— decomposition of urine, 190
Bacteriological methods, 311
Bacteriology, 312
Bacterium termo, 131
Bacteriuria, idiopathic, 190
Balantidium coli, 142
Bamberger's hæmatogenic albuminuria, 280
Barium salts, 101
Basic aniline dyes, 22, 27, 75, 326, 335
—— phosphate of magnesia, 197
—— phosphatic earths, 204
Basophile granules, 335
Baumann and *Udransky's* method, 120
Bence Jones on alkaline urine, 174
Benzoic acid in urine, 225
Benzo-purpurin, 99, 139, 321
—— test for HCl, 99
—— test-papers, 99
Benzoyl-chloride test for sugar, 48, 224, 230
—— for diamines, 120
—— for carbo-hydrates, 230
Beri-beri, 153
Berlin-blue reaction, 249
Betain, 260
Betol, 286
Bettelheim's granules, 24
Bidder and *Schmidt's* method for estimating HCl, 101
Bilberries, amylic alcohol extract of, 100
Bile acids in blood 49
—— acids in fæces, 160
—— acids in pus, 299
—— acids in urine, 237
—— acids, tests for, 49, 237
—— pigment in blood, 50
—— pigment in cysts, 303
—— pigment in fæces, 126, 164
—— pigment in pus, 299
—— pigment in semen, 307
—— pigment in urine, 173
—— pigment, tests for, 238
Bilifuscin, 238
Biliprasin, 238
Bilirubin, 39, 50, 196, 238, 303, 307
—— formula of, 40
Biliverdin in blood, 51
—— in fæces, 125
—— in sputum, 88
—— in urine, 238
—— in vomit, 108
Biology of micro-organisms, 312, 327
Bismarck-brown, 76
Biuret, 46
Biuret-test, 46, 104, 211
Bizzozero's chromo-cytometer, 12, 330
Bladder, calculus of, 277

Bladder, catarrh of, 276
—— epithelium of, 178
—— foreign bodies in, 277
—— tumours of, 277
Blennorrhœa, 177
Blood, the, 1
—— acetone in, 51
—— alkalinity of, 2, 4
—— animal parasites of, 35
—— ash of, 51
—— bile substances in, 49
—— chemical changes in, 37
—— colour of, 1
—— colouring matter of, 43
—— estimation of sugar in, 336
—— fatty acids in, 49
—— fibrin of, 45
—— formed elements of, 5
—— hæmoglobin of, 335
—— in anæmia, 25
—— in carcinoma, 48
—— in chlorosis, 25
—— in dyspnœa, 41
—— in fæces, 129, 167
—— in fever, 52
—— in melanæmia, 23
—— in poisoning, 41
—— in pus, 300
—— in sputum, 87, 91
—— in urine, 222
—— in vomit, 109
—— lactic acid in, 49
—— organic acids of, 49
—— parasites of, 27
—— peptones of, 45
—— proteids of, 45
—— protozoa in, 35
—— reaction of, 2, 329
—— sarcolactic acid in, 49
—— specific gravity of, 4, 329
Blood-colouring matter, relation of, to bile-pigment, 39
Blood-cells, *see* Blood-corpuscles and Leucocytes
Blood-corpuscles, red, apparatus for counting, 7
—— changes in, 6
—— counting of, 7, 8, 10
—— in buccal secretion, 53
—— in cysts, 302
—— in fæces, 129
—— in pus, 300
—— in sputum, 66
—— in transudations, 301
—— in urine, 175, 222
—— tests for, 222
Blood-pigment, 37
—— detection of changes in, 34
—— in fæces, 164
—— in gastric juice, 109
Blood-platelets, 5, 329
Blood-serum, lutein in, 43
—— spectrum of, 43
—— sterilised, 317
Blue milk, 309
Bodo urinarius, 193
Bothriocephalus cordatus, 146
—— latus, 145
—— liguloides, 146
Böttger's test, 227
β-oxy-butyric acid, 233
—— tests for, 280
Brandberg's method, 213
Bread in the nutrition of fungi, 320
Brieger's method for separation of ptomaines, 119
Bright on albuminuria, 206
Brilliant-green tests for HCl, 98
Bromide of potassium, detection of, in urine, 285
—— of potassium, detection of, in saliva, 57
Bromine salts in urine, 285
Bronchial asthma, 69
—— catarrh, 85
Bronchiectasis, 85
—— sputum of, 82
Bronchitis, acute, 85
—— chronic, 85
—— plastic, 86
—— putrid, 85
Broth, nutrient, 320
Browning's spectroscope, 43
Buccal secretion, 53
—— secretion, chemical constitution of, 55
—— secretion, ferment of, 55
—— secretion, fungi of, 53, 58
—— secretion, in disease, 56, 58
—— secretion, nitrites of, 56
—— secretion, physical characters of, 53
—— secretion, sulphocyanide of, 55, 57
Bujwid's staining process, 135
Bunsen's tables, 259
Butyric acid, detection of, 161
—— acid in fæces, 161
—— acid in gastric juice, 103
—— acid in sputum, 84
—— acid in urine, 251. *See also* Fatty acids

Cadaverin, 120, 263, 291
Calcium carbonate, crystals of, 157
—— phosphate in fæces, 157
—— phosphate, crystals of, 157, 197
—— sulphate, crystals of, 198
—— sulphate in fæces, 157
—— sulphate in urine, 198, 202
Calculus, 277
Calliphora erythrocephala, 155
Calomel, 114, 247
Canada balsam, 28
Cancer, blood in, 48
—— of alimentary canal, 173
—— of stomach, 110, 278
Capric acid, 162

Caproic acid in fæces, 162
—— acid in sputum, 84
—— acid in urine, 224
Carbol-fuchsin, 77
—— -gelatine, 139
Carbolic acid, estimation of, 244
—— acid in fæces, 162
—— acid in urine, 244, 284
—— acid in vomit, 122
—— acid poisoning, 122, 284
—— acid, tests for, 122
Carbohydrates in blood, 48
—— in fæces, 159
—— in gastric juice, 105
—— tests for, 48, 160, 224
Carbolo-chloride of iron test for lactic acid, 103
Carbonate of lime in fæces, 157
—— of lime in sputum, 84
—— of lime in urine, 203, 271
—— of magnesia in sputum, 84
Carbonates of the alkaline earths, 204
—— in sputum, 84
—— of urine, 271
Carbonic oxide, detection of, 41
—— oxide hæmoglobin, 41
—— oxide hæmoglobin, spectrum of, 41
—— oxide poisoning, 41, 285
Carburetted hydrogen in fæces, 164
Caries of teeth, 54
Casein in fæces, 128
—— in milk, 308
Casts, cellular, 181
—— chemistry of, 188
—— classification of, 180
—— "detritus," 180
—— examination of, 187
—— fatty, 185
—— granular, 183
—— hyaline, 186
—— metamorphosed, 180, 274
—— of micrococci, 181
—— organised, 180
—— staining of, 188
—— unorganised, 180
—— urinary, 180
—— waxy, 185
Cat, round worm of, 149
Catarrh, bronchial, 85
—— gastric, 108
—— nasal, 64
—— vesical, 276
Catarrhal urethritis, 277
Caustic potash, poisoning with, *see* Poisoning with alkalies
—— soda, poisoning with, *see* Poisoning with alkalies
Cellulose in blood, 48
Cercomonads in fæces, 141
—— in pus, 298
—— in sputum, 80
—— in urine, 193
Cercomonas intestinalis, 141
Cerebral hæmorrhage, urine in, 240
Cerebro-spinal fluid, 64
—— meningitis, 218
Certoneura stabulans, 155
Cestodes, 142
Charcot-Leyden crystals, 64, 70, 81, 152, 155
Cheese-maggot, 155
—— spirillum of, 137
Chemical examination of blood, 37
—— examination of buccal secretion, 55
—— examination of cystic fluids, 303
—— examination of fæces, 158
—— examination of gastric juice, 94, 106
—— examination of milk, 309
—— examination of pus, 298
—— examination of semen, 327
—— examination of sputum, 84
—— examination of transudations, 301
—— examination of urine, 205
—— examination of vomit, 111
Chinanisol, 287
Chinon-tyrosin reaction, 200
Chloral in urine, 225
Chlorate of potash in blood, 43
—— of potash, poisoning with, 43, 113
—— of potash, spectrum of, 43
Chloride of ammonium in urine, 265
—— of barium, 101
—— of gold test, 120
—— of magnesium in sputum, 84
—— of magnesium in urine, 265
—— of platinum test, 120
—— of potassium in urine, 265
—— of sodium in blood, 51
—— of sodium in fæces, 164
—— of sodium in urine, 265
—— of zinc solution, 261
Chlorides, estimation of, 265
—— in fæces, 164
—— in sputum, 84
—— in urine, 265
—— tests for, 265
Chloroform, poisoning with, 121, 284
—— tests for, 121
Chlorophyll, 127
Chlorosis, blood changes in, 25
—— urine in, 174
Cholæmia, 49
Cholecyanin test, 238
Choleglobin, 49
Cholera, Asiatic, 168
—— Asiatic, bacillus of, 133
—— infantum, bacillus of, 125
—— nostras, bacillus of, 137
Cholesterin crystals, detection of, 81
—— crystals in fæces, 156
—— crystals in pus, 298
—— crystals in sputum, 81
—— crystals in urine, 204
—— crystals, tests for, 163
Chololithiasis, 126
Choluria, 237
Chromocytometer, 12, 330
Chromogens of urine, 173, 248

Chromogens of urobilin, 173
Chromometer, 12, 333
Chronic bronchitis, 85
—— enteritis, 166
—— gastritis, 108
—— inflammation (non-tubercular) of lung, 87
—— nephritis, 274
Chrysophanic acid, 288
Chylous ascites, 343
—— exudation, 301
Chyluria, 252
Ciliated epithelium in cysts, 303
—— epithelium in sputum, 67
Cladothrix, 295
Claret, colouring matter of, 100
Claude Bernard's method, 336
Clostridium butyricum, 132
Coagulated albumin, 128
Coating of the tongue, 60
Coccidium perforans, 141
—— oviforme, 61
Coccus salivarius septicus, 54
Cochin-China diarrhœa, 154
Coffin-lid crystals, *see* Triple phosphate
Colour of blood, 1, 37
—— of exudations, 300
—— of fæces, 125
—— of sputum, 65
—— of transudations, 301
—— of urine, 172, 174, 340
—— scale for urine, 174, 340
—— tests for albumin, 212
Colouring matter of bile, 40, 164
—— matter of blood, 37
—— matter of fæces, 163
—— matter of urine, 173
Colostrum, 308
Comma-bacillus, 133
Compensation eye-piece, 314, 347
Compound sulphuric acids, 243, 245
Concretions of fæces, 127
—— of nasal secretion, 64
—— of urine, 202, 205
Condenser, *Abbe's*, 312
Congo-red test for HCl, 9
—— staining micro-organisms with, 139, 321
Connective tissue in sputum, 72
—— tissue in stools, 127
—— tissue in vomit, 107
Contracted kidney, 274
Copaiba balsam, 289
Copper, poisoning with salts of, 114, 282
Corpora amylacea in sputum, 72
Correction collars, 313
Cover-glasses, preparation of, 27, 76
Croton oil, 291
Croup, gastric, 111
Crystals, calcium carbonate, 157
—— calcium phosphate, 157, 197
—— calcium sulphate, 198
—— *Charcot-Leyden*, 64, 70, 81, 152, 155
Crystals, cholesterin, 81, 204, 298, 301
—— cystin, 198
—— fatty, 82, 156, 298
—— hippuric acid, 198
—— hæmin, 39
—— hæmatoidin, 81, 88, 129, 155, 196, 298
—— hæmoglobin, 26
—— in blood, 21
—— indigo, 203
—— in fæces, 157
—— in pus, 298
—— in sputum, 81
—— in urine, 195
—— leucin and tyrosin, 83, 199
—— magnesium phosphate, 197
—— margarine, 80, 82
—— oxalate of lime, 83, 157, 195
—— phenyl-glucosazon, 227
—— *Roussin's*, 118
—— spermatic, 307
—— sulphide of bismuth, 157
—— *Teichmann's*, 39
—— triple phosphate, 83, 157, 197, 202, 298, 338
—— tyrosin, 83
—— urate of ammonia, 204
—— uric acid, 195
—— xanthin, 199
Crystalline sediment of urine, 175
Cultivation, cylinder-, 323
—— drop-, 325
—— in depth of nutrient substances, 324
—— on glass slides, 325
—— on surface of nutrient substances, 325
—— plate-, 322
Curschmann's spirals, 69, 85
—— spirals, *Troup* on, 81
Cutaneous inoculation, 326
Cyanotic induration of kidneys, 184
Cylinder-cultivations, 323
Cylindroids, 181, 187
Cupric oxide, reduction of, 225
Cystic kidney, 304
Cystin, 205
Cystinuria, 253
Cystitis, 270
Cysts, colloid, 303
—— contents of, 302
—— dermoid, 303
—— hydatid, 302
—— of broad ligament, 303
—— ovarian, 302
Cytometer, 12, 331

Dahlia, acid solution of, 336
Damar varnish, 28
D'Arsonval's incubator, 324
Deneke's spirillum, 137
Deposit on teeth, 59
Dermatitis, urine in, 221
Dermoid cysts, 303
"Detritus" casts, 180
Detritus in fæces, 130

Deutero-albumose, 339
Dextrin in fæces, 160
—— in urine, 237
Dextrose, *see* Grape-sugar
Diabetes, blood in, 48
—— insipidus, 279
—— mellitus, 280
Diabetic acetonuria, 249
Diacetic acid, tests for, 250
Diaceturia, 250
Diamines in urine, 120
—— tests for, 263
Diaphragms, 347
Diastase, 164
—— in urine, 264
Diastatic ferment of cysts, 304
—— ferment of fæces, 164
—— ferment of saliva, 55
—— ferment of urine, 264
Diathesis, uric acid, 253
Diazobenzol, 121
Diazobenzol-sulphonic acid, 273
Diazo reaction, *Ehrlich's*, 273
Dickinson on coating of tongue, 60
Differentiation of proteids in urine, 207
Digestion, stages of, 104
Digitalis, 171
Dilatation of stomach, 108
Dioxyquinin, 286
Diphtheria, bacillus of, 61
—— gastric, 111
Diplococci in ozæna, 64
—— in pneumonia, 78
—— in pus, 291
Dipteral larvæ, 64, 155, 339
Disseminated sarcoma, 218
Distoma eggs in blood, 36
—— eggs in urine, 193
—— hæmatobium, 36
—— hæmatobium in blood, 35
—— hæmatobium in sputum, 80
—— hæmatobium in urine, 193
—— hepaticum, 147
—— lanceolatum, 147
—— Rathonisi, 148
Dochmius duodenalis, 150
Double sulphate of iron and ammonia solution, 266
Dragendorff's test for bile-pigment, 239
Dreschfeld's test for HCl, 339
Drop-cultivations, 325
—— of cholera-bacillus, 135
Drosophila melanogastra, 155
Dry measurement, 25
Duodenal ulcer, 110
Dysentery, 167
Dyspepsia, acid in, 96
—— urine in, 278
Dyspnœa, blood changes in, 41

EARTHY phosphates, 204, 268
Echinococcus in sputum, 80
—— in urine, 193
—— cysts in sputum, 80
Echinococcus cysts in urine, 193
—— hooklets from cysts, 302
—— hooklets in fæces, 80
—— hooklets in gastric juice, 111
—— hooklets in urine, 193
Ehrlich's granules, 22, 335
—— staining method, 27
—— test for bilirubin, 239
—— test for typhoid urine, 273
Ehrlich-Weigert fluid, 28, 76
Eggs of helminthæ in blood, 36
—— of helminthæ in fæces, 152
—— of helminthæ in sputum, 80
—— of helminthæ in urine, 193
Elastic fibres in fæces, 127
—— fibres in gastric juice, 107
—— fibres in sputum, 68, 87
Embolic lipæmia, 49
—— nephritis, 182
Emerald-green test for HCl, 98
Emphysema, urine in, 173
Endocarditis, 191
Enteritis, acute, 165
—— chronic, 166
—— membranacea, 126
—— mucosa, 126
Entero-colitis, monadines in, 140
Enterogenic peptonuria, 218
Entozoa in nose, 64
Eosin, 22, 188, 335
Eosinophile granules, 22, 335
Epileptic urine, casts in, 186
—— urine, spermatozoa in, 188
Epithelium, alveolar, 67, 89
—— ciliated, 67, 303
—— in buccal secretion, 53
—— in exudations, 291
—— in fæces, 129
—— in gastric juice, 93
—— in nasal secretion, 63
—— in sputum, 67
—— in transudations, 301
—— in urine, 177
—— in vaginal secretion, 309
—— obsolete in fæces, 130
—— of male urethra, 178
—— renal, 179
—— vesical, 178
Eristalis arbustorum, 155
Erysipelas, streptococcus of, 33, 191
Erythro-dextrin, test for, 105
Esbach's albuminimeter, 216
Esmarch's cylinder-cultivations, 323
Eustrongylus gigas, 194
Ewald's test for HCl, 97
Exogenic toxicosis, 264
Experimental albuminuria, 206
Exsiccator, 22
Exudations, 290
—— chylous, 301
—— hæmorrhagic, 300
—— purulent, 290
—— putrid, 299
—— sero-purulent, 299

Exudations, serous, 300
—— *see also* Pus
Eye-piece, 314
—— periscopic, 314

Fæces, the, 125
—— blood in, 129, 167
—— chemistry of, 157
—— colour of, 125
—— colouring matters of, 163
—— crystals of, 155
—— detritus of, 130
—— fats in, 127
—— ferments in, 164
—— foreign bodies in, 127
—— formed elements of, 129
—— fungi of, 131
—— hydatids in, 146
—— in diseases of the alimentary canal, 165
—— insects in, 155
—— microscopical characters of, 127
—— mucin in, 127
—— naked-eye characters of, 125
—— non-pathogenic fungi of, 131
—— parasites of, 130
—— pathogenic fungi of, 133
—— proteids of, 128
—— quantity of, 126
—— reaction of, 125
—— worms in, 142
Fat crystals, 156
—— crystals in fæces, 127, 156
—— crystals in pus, 299
—— crystals in sputum, 82
—— crystals in vaginal secretion, 310
—— crystals in urine, 202
—— crystals in vomit, 107
—— crystals, separation of, 84
Fat in blood, 49
—— in fæces, 127
—— in sputum, 82
—— in urine, 202
Fatty acids in urine, 251
—— acids, non-volatile, of fæces, 162
—— acids of sputum, 84
—— acids, tests for, 251
—— acids, volatile of blood, 49
—— acids, volatile, of fæces, 160
—— casts, 185
—— needles, *see* Fat crystals
Favus of stomach, 111
Febrile acetonuria, 249
—— albuminuria, 208
—— states, urine of, 343
Fehling's fluid, 48, 231
—— titration method, 230
Ferment, diastatic, 264
—— of fæces, 164
—— milk-curdling, 94
—— of saliva, 55
—— of sputum, 84, 91
—— of urine, 264
Fermentation test for sugar, 226
Ferments of gastric juice, 94, 265
Ferments of urine, 264
Ferric salts in sputum, 84
Ferrocyanide, 265
Fibres, elastic, in fæces, 127
—— elastic, in gastric juice, 107
—— elastic, in sputum, 68, 87
Fibrin, estimation of, in blood, 45
—— estimation of, in exudations, 299
—— estimation of, in urine, 222
—— of blood, 45
Fibrinous coagula in sputum, 71
—— coagula in urine, 222
Fibrinuria, 207, 222
Filaria inermis, 298
—— sanguinis hominis, 36, 193
Filariæ in blood, 36
—— in pus, 398
—— in urine, 193
Finkler-Prior bacillus, 137
Fission-fungi of blood, 26
—— of fæces, 131
—— of sputum, 74
—— of vaginal secretion, 310
—— of vomit, 108
Fistulæ, secretions from, 304
Fluke, 147
Foreign bodies in fæces, 127
—— bodies in sputum, 67
—— bodies in urine, 205
Formic acid, detection of, 161
—— acid in blood, 49
—— acid in fæces, 161
—— acid in gastric juice, 99
—— acid in urine, 251
—— acid reaction in gastric juice, 98
Fränkel's pneumono-coccus, 78
Fresenius and *Babo's* test for lead, 113
Friedländer's method for staining fungi, 78
—— pneumono-coccus, 78, 89
Fröhde's reagent, 117
Fruit-sugar, *see* Lævulose.
Fuchsin for staining micro-organisms, 28
—— test for HCl, 98
Fungi in ozæna, 64
—— of blood, 27
—— of buccal secretion, 53, 58
—— of fæces, 130
—— of nasal secretion, 63, 64
—— of pus, 291
—— of serous exudation, 300
—— of sputum, 73
—— of urine, 189
—— of vaginal secretion, 310
—— of vomit, 108
—— spores of, 31, 59, 324. *See also* Micro-organisms
Fungoid plugs, 74, 85
Fürbringer's test, 211
Furfurol, 46, 49, 50, 108
—— test for bile acids in urine, 237
—— test for carbohydrates, 48, 230
—— test for urea, 46
Fusiform degeneration, 129

Gabbett's staining method, 338
Gangrene of lung, 84, 91
Gangrenous stools, 167
Gases in blood, 41
—— intestinal, 164, 339
—— of urine, 272
Gastric croup, 111
—— diphtheria, 111
Gastric juice, acetic acid in, 103
—— juice, acidity of, 96
—— juice, acids of, 95
—— juice, ammonia salts in, 105
—— juice and vomit, 93
—— juice, butyric acid in, 103
—— juice, carbohydrates in, 105
—— juice, chemical examination of, 106
—— juice, chemistry of, 94
—— juice, excessive secretion of, 96
—— juice, formed elements of, 93
—— juice, hyperacidity of, 96
—— juice, lactic acid in, 103
—— juice, method of obtaining, 93, 94
—— juice, milk-curdling ferment of, 95
—— juice, naked-eye characters of, 93
—— juice, organic acids of, 103
—— juice, pepsin of, 94
—— juice, proteids of, 103
—— juice, urea in, 105
—— juice, zymogen of, 95
Gastric sound, 93
—— ulcer, 96, 109
Gastritis, acute, 108
—— atrophic, 109
—— chronic, 108
Gelatine, nutrient, 319
Genital organs, secretions of, 306
Gentian-violet, 28, 75
—— aniline-water solution, 28, 31, 75
Giacomi's method for staining micro-organisms, 293
Giant-corpuscles, 291
Gibbes' staining method, 337
Glanders, bacillus of, 32
—— bacillus of, in blood, 32
—— bacillus of, in pus, 296
—— bacillus of, in urine, 191
Globulin in pus, 298
—— in semen, 308
—— in urine, 207, 221
—— test for, 221
Globulinuria, 207, 221
Glycerine in urine, 225
Glycogen in exudations, 299
—— in sputum, 84
Glycogenic reaction, 177
Glycosuria, 224
—— pathological, 224
—— persistent, 224
—— physiological, 224
—— transitory, 244
Glycuronic acid phenomenon, 287
Gmelin's test, 238
Goblet-cells in fæces, 129
Gonococci, 277
Gonorrhœal urethritis, 277
Gout, blood in, 47
—— urine in, 256
Gowers' hæmacytometer, 11
Gräber's solution, 8
Gram and *Gräber's* theory of microcytes, 24
Gram's staining method, 28
Granular casts, 182
Granules in colourless blood-corpuscles, 22, 335
Grape-sugar, tests for, 225. *See* Sugar
Grass-green sputum, 88
Green stools, 125
Griess' papers, 337
Guanidin carbonate, 161
Guanimin of isobutyric acid 161
Günther's staining method, 78
Gunzburg's reagent, 101

Hæmacytometer, 11
Hæmatemesis, 109
Hæmatin, formula of, 40
—— in vomit, 110
—— reduced, 40
—— spectrum of, 40
Hæmatogenic albuminuria, 208
—— jaundice, 238
—— peptonuria, 217
Hæmatoidin, amorphous, 26
—— crystals, 39, 81, 88, 129, 155, 196, 291, 298
Hæmatophyllum malariæ, 35
Hæmato-porphyrin, 39
Hæmatoscope, 15
Hæmatospectroscope, 17
Hæmatoxylin solution, 188
Hæmatozoa, 35
Hæmaturia, 207, 222
—— intermittent, 176
Hæmin-crystals, 39. *See Teichmann's* crystals
Hæmoglobin, amount of, 335
—— changes in, 1
—— proteid of, 38
—— reduced, 38
—— rhombic crystals of, 26
—— spectrum of, 38
Hæmoglobinæmia, 43
Hæmoglobinuria, 43, 207, 223
—— paroxysmal, 223
Hæmometer, 13
Hæmoptysis, 87, 91
Hæmorrhagic exudation, 300
—— infarction of lung, 92
—— sputum, 87, 91
—— stools, 108
Hairs in urine, 205
Hay-bacillus, 139
Haycraft and *Williamson's* test for alkalinity of blood, 4
Hayem's solution, 5
Heat-apoplexy, 168
Heller's test, 211

Helminthiasis, 142
Hemialbumose, 104, 109, 339
Hen-cholera, bacillus of, 315
Hénocque's double spectroscope, 17
—— formula, 19
—— hæmatoscope, 15
—— hæmato-spectroscope, 17
Hepatic diseases, fæces in, 169
—— diseases, urine in, 278
Hepatogenic jaundice, 238
Hering's lensless spectroscope, 44
Hetero-albumose, 339
Hexahydro-hæmatoporphyrin 39
Heynsius' test, 211
Hindenlang's test, 211
Hippuric acid, 198
Hofmeister's test for peptone, 219
Homogeneous immersion lens, 313
Homolomyia, 155
Hufner's apparatus, 257
Huppert's test for bile-pigments, 50, 239
Hyaline casts, 186
Hydatid cysts, 302
Hydatids in fæces, 146
—— in sputum, 80
Hydræmia, 45
Hydriodic acid, 175
Hydrobilirubin in fæces, 126
Hydrochinon, 173, 244
Hydrochlorate of phenyl-hydrazin, 226
Hydrochloric acid, benzo-purpurin test for, 99
—— acid, bilberries, test for, 100
—— acid, Congo-red test for, 98
—— acid, detection of, 96
—— acid, *Dreschfeld's* test for, 339
—— acid, emerald-green test for, 98
—— acid, estimation of, 101
—— acid, *Ewald's* test for, 97
—— acid, fuchsin test for, 97
—— acid, in cancer of stomach, 110
—— acid, in gastric juice, 96
—— acid, methyl-aniline-violet test for, 97
—— acid, *Mohr's* test for, 97
—— acid, oo-trapæolin test for, 97
—— acid, phloro-glucin and vanillin test for, 98
—— acid, *Uffelmann's* tests for, 100
—— acid, ultramarine and zinc sulphide test for, 100
Hydrocyanic acid poisoning, 42
Hydronephrosis, 304
Hydroparacumaric acid, 244
Hydrothæa meteorica, 155
Hydrothionæmia, 42
Hydrothionuria, 271
Hyperacidity of gastric juice, 96
Hypersecretion of gastric juice, 96
Hypopyon, 291
Hyposulphites of urine, 267
Hyposulphurous acid in urine, 271
Hypoxanthin, 260, 262
Hysteria, 205

Idiopathic bacteriuria, 190
—— oxaluria, 253
Incubator, 324
Indican, 173, 242, 278
—— estimation of, 243
—— tests for, 242
Indicanuria, 241
Indigo, 203, 241
—— -blue, 241
—— -carmine test-paper, 228
Indigotin, 241
Indirubin, 242
Indol in fæces, 162
—— in urine, 249
Indoxyl-sulphate, 203
—— -sulphuric acid, 241
Indulin, 22
Infarction, hæmorrhagic, of lung, 92
Infectious diseases, blood in, 24
—— diseases, urine in, 179, 391
Inflammation, non-tubercular, of lung, 87
Infusoria in fæces, 141
—— in pus, 298
—— in sputum, 80
—— in urine, 192
—— in vaginal secretion, 310
Inoculation with micro-organisms, 326
Inorganic substances in blood, 51
—— substances in fæces, 164
—— substances in semen, 307
—— substances in sputum, 84
—— substances in urine, 265
Inosite in cysts, 302
—— test for, 237
Inosituria, 237
Insects in fæces, 155, 339
Intermittent albuminuria, 208
—— hæmaturia, 175
Intestinal catarrh, 165
—— gases, 164, 339
—— putrefaction, 242
—— ulcer, 221
Intestines, the, diseases of, 165
—— tuberculosis of, 166
Invertin, 164
Iodates, 285
Iodine in urine, 285
—— test for, 285
Iodo-ammonic-iodide, 131
Iodoform in urine, 285
—— test for, 285
Iodo-potassic-iodide test for micro-organisms, 132
Iris diaphragm, 347
Iron, salts of, in sputum, 84
Iso-butyric acid, 161
Iso-cyan-phenyl test, 122

Jacquemin's test for nitrobenzol, 121
Jaffe's test for indican, 242
—— for kreatin, 261
Jaundice, 169
—— casts in, 180
—— hæmatogenic, 238

Jaundice, hepatogenic, 238
—— inogenic, 238
—— urobilin, 240
Johnson's picric acid test for sugar, 228

Kairin in urine, 173, 286
—— potassium sulphate, 286
Kidney, amyloid, 179, 275
—— caseation of, 192
—— contracted, 275
—— cyanotic induration of, 184
—— cystic, 304
—— epithelium of, 179
—— large white, 186
—— small red, 275
Klebs and *Tommasi-Crudeli's* bacillus of malaria, 35
Klemperer on thrush fungus, 59
Knife-rest crystals, *see* Triple phosphate
Koch and *Ehrlich's* staining methods, 75
Koch's pure cultivations, 321
Kreatin, 225
Kreatinin, 225
Kreatinin-zinc chloride, 225
Kühne's staining methods, 315

Lactic acid bacillus, 165
—— acid, detection of, 280
—— acid fermentation, 108
—— acid in blood, 49
—— acid in gastric juice, 102
—— acid reaction in gastric juice, 98
—— acid, tests for, 103
Lactosuria, 237
Lævulosuria, 236
Landois' test for reaction of blood, 2
Larvæ, dipteral, 64, 155, 339
Lassar's test for reaction of blood, 2
Latent disseminated sarcoma, 218
Lead colic, 281
Lead, poisoning with salts of, 113, 281
Lecithin corpuscles, 307
Legal's test for acetone, 249
Lensless spectroscope, 44
Leprosy, bacillus of, 297
Leptothrix buccalis, 54, 59
—— buccalis in sputum, 74, 91
Leube and *Salkowski's* titration process, 231
Leucin in sputum, 83
—— in urine, 199, 279, 280
Leucocytes in blood, 19
—— in exudations, 291
—— in fæces, 129
—— in semen, 307
—— in sputum, 66
—— in urine, 176
—— in uterine secretion, 310
—— in vomit, 107
Leucocytosis, 6, 20
Leucohypobilin, 169
Leucomaines, *see* Ptomaines
Leucourobilin, 169
Leukæmia, 6
Leukæmia crystals, 21
—— lymphatico-splenic, 21
—— myelogenic, 21
—— splenic, 21
Lichtheim's plate, 322
Lieben's test for acetone, 250
Lipacidæmia, 49
Lipaciduria, 251
Lipæmia, 49
Lippich's polarimeter, 233
Lipuria, 251
Litmus, extract of, 295
Liver, diseases of, fæces in, 169
—— diseases of, urine in, 278
Lochia, the, 310
Löffler's staining process, 28
Lowenberg's diplococcus, 64
Lucilia cæsar, 155
—— regina, 155
Ludwig's filter, 255
—— process, 254
Lumniczer's bacillus, 85
Lung, *see* Pulmonary
Lustgarten's bacillus, 293
Lutein, spectrum of, 43
Lymphatico-splenic leukæmia 21

Mackay's test for bile-acids, 50
Magenta solution, 338
Magnesia mixture, 254
—— soaps of, in fæces, 156
Magnesium phosphate, 197
Malaria, micro-organisms in, 34
Maltose in urine, 237
Maly's test for HCl, 97
Mammary secretion, 308
Margarine needles in sputum, 82
Mason's lung, 92
"Mastzellen," 336
Measles, urine test for, 273
Measurement of urine, 170
Meconium, 165
Megaloblasts, 23
Megastoma entericum, 141
Melanæmia, 23
Melanin, 228
—— tests for, 248
Melanogen, 228, 248
Melanuria, 248
Melithæmia, 47
Menstruation, 310
Mercury, poisoning with salts of, 113, 281
—— tests for, 114, 282
Metadiamidobenzol, 271
Metalbumin in cysts, 303
Metamorphosed casts, 180, 274
Metaphosphoric acid test for albumin, 211
Metatungstic acid, 120
Methæmoglobin in blood, 40, 43
—— in urine, 223
—— spectrum of, 40
Methan, 164

Methyl-aniline-violet test for HCl, 97
—— for staining fungi, 28
Methylene blue, 28, 272
Methyl-violet, 75
Microbes in pneumonic sputum, 89
—— tests for, 303
Micrococci in buccal secretion, 60
—— in nasal secretion, 64
—— in pus, 291
—— in urine, 189
—— *see also* Fungi
Micrococcus chlorinus, 88
—— *de la rage*, 54
—— erysipelatos, 191
—— gonorrhoicus, 279
—— of sputum-septicæmia, 54
—— prodigiosus, 309
—— tetragenus, 54
—— ureæ, 174, 189
Microcytes, 23
Microcythæmia, 6, 23
Micro-organisms, detection of, 314
—— examination of blood for, 27
—— in hydrophobia, 34
—— in malaria, 34
—— in scarlatina, 33
—— in small-pox, 35
—— methods of staining, 27, 75
—— of fæces, 131
—— of milk, 308
—— of mouth, 53, 58
—— of nasal secretion, 64
—— of pus, 291
—— of urine, 189
—— of vaginal secretion, 310
—— of vomit, 108
—— spores of, 31, 69, 324
—— transmission of, to animals, 325
—— *see also* Fungi
Microscope, the, 312
Microscopical examination of the blood, 5
—— examination of cystic fluids, 302
—— examination of exudations, 291
—— examination of the buccal fluids, 53
—— examination of the fæces, 127
—— examination of the gastric contents, 93
—— examination of the nasal secretion, 63
—— examination of the semen, 306
—— examination of the sputum, 66
—— examination of the urine, 174
—— examination of the vomit, 107
—— examination of transudations, 301
—— examination of vaginal secretion, 309
Miliary tuberculosis of lung, 86
—— tuberculosis of urinary organs, 277
Milk, the, 358
—— -curdling ferment in gastric juice, 94
—— -curdling ferment in urine, 265
—— -sugar, 237
Millon's reagent, 161, 212
Mohr's estimation of chlorides, 265
—— tests for HCl, 97
Mölisch's sugar reactions, 229
Molybdate of soda, 117
Monadines in fæces, 140
—— in sputum, 91
Monads in fæces, 141
—— in sputum, 80
Monilia candida, 59
Monohydroxyl-benzol derivatives, 212
Moore's test for sugar, 48, 225
Mordants in staining micro-organisms, 337
Morphia in urine, 283
—— poisoning, 117, 283
—— tests for, 117
Mould fungi of blood, 26
—— fungi of fæces, 130
—— fungi of gastric juice, 108
—— fungi of mouth, 53
—— fungi of nose, 64
—— fungi of sputum, 73
—— fungi of urine, 189
—— fungi of vaginal secretion, 310
—— fungi of vomit, 108
Mucin in fæces, 158
—— in nasal secretion, 63
—— in saliva, 55
—— in sputum, 84
—— in urine, 207, 223
—— tests for, 223
Mucinuria, 207, 223
Mulder's test, 228
Murexide test, 47
Muscle, trichina of, 153
—— fibres in fæces, 127
—— fibres in vomit, 107
Myelin droplets, 68
Myelogenic leukæmia, 21

Naphthaline in urine, 288
Naphthalol, 286
Nasal catarrh, 64
—— secretion, 63
—— secretion, cerebro-spinal fluid in, 64
—— secretion, characters of, 63
—— secretion, *Charcot-Leyden* crystals in, 64
—— secretion, concretions in, 64
—— secretion in disease, 63
—— secretion, micro-organisms of, 63, 64
—— secretion, pus in, 64
Nematodes, 148
Nephritis, 274
—— acute, 274
—— chronic, 274
—— embolic, 182
—— gravidarum, 196
—— sp. gr. of urine in, 172
—— toxic, 281
Nephrolithiasis, 205
Neubauer's method, 252
Neuralgia, saliva in, 56
Neutral phosphate of lime in fæces, 157

Neutral phosphate of lime in urine, 197
—— phosphates, 268
Neutrophile granules, 335
Nitrate of silver solution, 266
Nitrates in urine, 271
Nicotin, poisoning with, 118, 283
Nitric acid, poisoning with, 112
—— acid test for albumin, 210
Nitrites in the blood, 43
—— in the saliva, 56
—— in the urine, 271
—— tests for, 271, 337
Nitrobenzol, poisoning with, 43, 122
—— tests for, 123
Nitrogen, estimation of, in urine, 260
Nitro-prusside test, 124
—— of sodium as a test for acetone, 249
—— of sodium as a test for melanuria, 248
Normal acid solution, 3
—— salt solution, 9
—— soda solution, 12
—— urobilin, 341
Non-pathogenic fungi of blood, 26
—— fungi of fæces, 130
—— fungi of gastric juice and vomit, 108
—— fungi of pus, 291
—— fungi of sputum, 73
—— fungi of urine, 189
—— fungi of vaginal secretion, 310
Nose, secretion from, 63
Nose-piece of microscope, 346
Nosotoxicosis, 263
Nuclein, 299
—— in milk, 308
—— in pus, 299
—— in semen, 308
—— in sputum, 84
Nutrient agar-agar, 320
—— blood serum, 319
—— fluids, 319
—— fluids, sterilisation of, 317
—— gelatine, 319
—— gluten, 320
—— potato, 320
—— starch, 320
—— substances, 318
—— substances, stained, 321
—— substances, sterilisation of, 317
Nylander's test for sugar, 228

Objectives, 313
—— apochromatic, 314
Œdema, pulmonary, 91
Oïdium albicans, 55
Oil-immersion lens, 313
Oil of cloves, 28
Oleic acid, 162
Oligochromæmia, 8
Oligocythæmia, 7
Oliguria, 171
Omichol, 262
Optimum temperature, 318
Orchitis, 307
Organic acids of blood, 49
—— acids of fæces, 161
—— acids of gastric juice, 103
—— acids of sputum, 84
—— acids of urine, 251
—— salts of lime in fæces, 157
Organised sediment of urine, 175
—— casts in, 180
Osteomalacia, 52, 221
Ovarian cysts, 302
—— cysts, in fæces, 157
—— cysts, in sputum, 88
—— cysts, in urine, 195, 201
Oxalate of lime, crystals of, 83, 157, 195
Oxalic acid diathesis, 252
—— acid, estimation of, 252
—— acid in urine, 196, 252
—— acid, poisoning with, 112
Oxaluria, 196, 252
—— idiopathic, 253
—— vicarious, 253
Oxyacids, aromatic, 244
Oxyamygdalic acid, 279
Oxyhæmoglobin, spectrum of, 38
—— reduction of, 19
Oxyuris vermicularis, 150
Ozæna, 64
Ozone, 272

P-amido-dimethyl-aniline, 272
Pacini's solution, 8
Palmitic acid, 162
Paraamido-phenol-sulphuric acid, 285
Parakresol, 244
Parakresol-æther-sulphuric acid, 244
Paralbumin, 303
Paramœcium coli, 142
Parasites of blood, 26
—— of buccal secretion, 54
—— of fæces, 130
—— of gastric juice and vomit, 108
—— of milk, 308
—— of nasal secretion, 64
—— of pus, 291
—— of sputum, 73
—— of urine, 189
—— of vaginal secretion, 310
Parasitic affections of stomach, 111
Paroxyphenyl-acetic acid, 244
—— -propionic acid, 244
Paroxysmal hæmoglobinuria, 223
Pathogenic fungi of blood, 26
—— fungi of buccal secretion, 54
—— fungi of fæces, 130
—— fungi of gastric contents, 108
—— fungi of milk, 308
—— fungi of nose, 64
—— fungi of pus, 291
—— fungi of sputum, 75
—— fungi of urine, 190
—— fungi of uterine secretion, 310
Pathological albuminuria, 206
—— acetonuria, 249
—— glycosuria, 224

Pathological urobilin, 343
Pentamethylene-diamine, 120, 263
Penzoldt's test, 228
Pepsin, detection of, 94
—— estimation of, 95
—— in gastric juice, 94
—— in urine, 264
—— in vomit, 110
Peptone in blood, 45
—— in fæces, 159
—— in gastric juice, 104
—— in pus, 299
—— in sputum, 84
—— in urine, 207, 217
—— tests for, 219
Peptonuria, 207, 217
—— enterogenic, 218
—— hæmatogenic, 217
—— puerperal, 218
—— pyogenic, 217
—— syphilitic, 219
Periscopic eye-piece, 314
Peritonitis, 159
Pernicious anæmia, 25
—— urine in, 174
Peroxide of hydrogen in urine, 272
Persistent glycosuria, 224
Pettenkofer's test, 50
Phantom corpuscles, 175
Pharyngomycosis leptothricia, 61
Phenacetin, 287
Phenol, estimation of, 244
—— in fæces, 162
—— in urine, 244
—— tests for, 162
—— -æther-sulphuric acid, 244
Phenols, estimation of, 246
Phenyl-glucosazon, 48, 227
—— -hydrazin chloride, 48, 226
—— -lactosazon, 237
Phlegmon, 290
Phloro-glucin, 98
Phloxin-red, 139
Phthisis, acute, 86
—— chronic, 87
Phosphates, estimation of, 269
—— in fæces, 157
—— in sputum, 84
—— in urine, 268
—— tests for, 269
Phosphatic sediment, 194, 197, 202
Phosphomolybdic acid test, 120
Phosphoric acid, estimation of, 269
Phosphorus poisoning, 116, 283
Phospho-tungstic acid, preparation of, 219
—— acid test, 120
Physiological acetonuria, 249
—— albuminuria, 206
—— glycosuria, 224
Picrate of indol, 249
Picric acid test for albumin, 212
—— acid test for sugar, 228
Picro-carmine, 188
Pilimictio, 205
Pilocarpine, 56
Pimaric acid, 210
Pinic acid, 210
Piophila casei, 155
Plasma of blood, 1
Plasmodium malariæ, 33
Plastic bronchitis, 71
Plate cultivations, 322
Platinum chloride test, 120
Platodes, 142
Pneumaturia, 205
Pneumoconiosis, 92
Pneumonia, 88
—— fibrinous coagula in, 71
—— micro-organisms of, 78, 89
—— sputum of, 78, 84, 88
Pneumonococcus, detection of, 78
—— of *Fränkel*, 78
—— of *Friedländer*, 78, 79
Poikilocytes, 25
Poikilocytosis, 6, 25
Poison elaborated by cholera bacillus, 136
Poisoning with acids, 111, 281
—— with alkalies, 112, 281
—— with alkaloids, 117, 283
—— with aniline, 43, 285
—— with carbolic acid, 283
—— with carbonic oxide, 41, 224, 285
—— with chlorate of potash, 42, 113
—— with chloroform, 121, 283
—— with ethylic alcohol, 121, 283
—— with hydrocyanic acid, 42, 123
—— with metals and metalloids, 113, 281
—— with nitro-benzol, 43, 285
—— with nitrous oxide, 41, 285
—— with ptomaines, 118, 283
—— with sulphuretted hydrogen, 42
Polarimeter, 233
Polarisation, 233
Pollenia rudis, 155
Polyuria, 171, 279, 280
Ponceau, 335
Potato, sterilised, 317
Pravaz' syringe, 326
Preparation of cover-glasses, 27
Propeptone in gastric juice, 109, 337
—— in semen, 221
—— in urine, 220
—— tests for, 221
Prostatic fluid, 306
Proteids formed in gastric digestion, 339
—— of blood, 45
—— of buccal secretion, 55
—— of cystic fluids, 303
—— of fæces, 128
—— of gastric juice, 108, 339
—— of pus, 298
—— of sputum, 84
—— of transudations, 301
—— of urine, 205, 342
—— tests for, 104

Proto-albumose, 339
Protozoa of blood in small-pox, 35
—— of fæces, 140
—— of sputum, 79
Prune-juice sputum, 84, 88
Prussic acid poisoning, 42
—— acid, tests for, 123
Psorospermia, 141
Ptomaines, detection of, 119
—— in blood, 51
—— in fæces, 164
—— in gastric juice, 118
—— in urine, 262, 283
—— poisoning with, 118
—— tests for, 120
Ptomato-atropin, 121
Ptyalism, 55
Puerperal peptonuria, 218
Pulmonary affections, sputum in, 85
—— abscess, 82, 90
—— gangrene, 91
—— œdema, 91
Pure cultivations, preparation of, 321
Purulent exudations, chemical examination of, 298
—— exudations, crystals in, 298
—— exudations, fungi of, 291
—— exudations, naked-eye characters of, 290
Pus, characters of, 290
—— chemistry of, 298
—— crystals of, 298
—— fungi of, 291
—— in fæces, 129
—— in nasal secretion, 64
—— in sputum, 66
—— in urine, 177
Putrefaction bases, 262
Putrescin, 120, 263
Putrid bronchitis, 85
—— exudation, 299
Pycnometer, 171
Pyelitis calculosa, 276
Pyelo-nephritis, casts in, 182
Pyogenic peptonuria, 217
Pyrocatechin, 244
—— separation of, 247
—— tests for, 247
Pyuria, 177

Quincke's inogenic jaundice, 238
Quinine in urine, 173, 286

Reaction of blood, 2
—— of fæces, 125
—— of gastric juice, 96
—— of pus, 290
—— of sputum, 65
—— of urine, 174, 341
Red blood-corpuscles, *see* Blood-corpuscles
Reduced hæmatin, 39
Reduced hæmoglobin, 38
—— hæmoglobin, spectrum of, 38
Reducin, 263
Reichert's lenses, 313
Relapsing fever, spirillum of, 30, 191
Renal albuminuria, 207
—— casts, 180
—— colic, 205
—— epithelium, 179
Resorcin in urine, 173
Retention-toxicosis, 263
Reynolds' test for acetone, 250
Rhabditis genitalis, 194
Rhabdonema strongyloides, 154
Rheumatic arthritis, urine in, 256
Rhinoliths, 64
Rhizopoda, 140
Rhubarb in urine, 173, 227
Ribbert's small red kidney, 275
Rice-water stools, 168
Richet's method for estimation of HCl, 101
Rickets, 82
Roberts' fermentation process, 232, 343
—— test for serum-globulin, 343
Rochelle salts, 231
Rosaniline hydrochloride, 337
Rosenbach's test for bile pigments, 239
Round worms, 148
Roussin's crystals, 118
Rubner's test for sugar, 229

Saccharomyces cerevisiæ, 108
—— ellipsoideus, 108, 131
Saccharomycetes, 74, 108, 130
Safranin, 188
Sago-grain forms in fæces, 126
Sahli's test for pepsin, 264
Salicylates in urine, 286
Saliva, chemistry of, 55
—— fungi of, 53
—— in disease, 56
—— microscopical appearances of, 53
—— naked-eye characters of, 53
—— *see also* Buccal secretion
Salivary corpuscles, 53
Salkowski's colorimetric process, 243
Salol, 286
Salts of the blood, 51
Santonin, 288
Sarcina of urine, 190
—— pulmonis, 74
—— ventriculi, 108
Sarcolactic acid in blood, 49
—— acid in urine, 281
Sarcophaga hæmatodes, 155
—— hæmorrhoidalis, 155
Sarkin, 260
Scarlatina, nephritis in, 179
—— streptococcus of, 33
Scatol, 162
Scheme of a bacteriological investigation, 326
Scherer's test for phosphorus, 116
Schottelius' method of cultivation, 135
Schreiner's base, 81, 306
Scolices, 302

Scurvy, urine in, 174
Searchers, 347
Sediment, urinary, 175
Seegen's method for testing for grape-sugar, 337
Semen, chemistry of, 307
—— physical characters of, 306
Senna in urine, 173
Sepsis occulta, 218
Septicæmia of mouse, bacillus of, 132
—— urine in, 191
Seropurulent exudation, 299
Serous exudation, 300
Serum-albumin in pus, 298
—— -albuminuria, 207
—— -globulin in urine, 222, 342
—— -globulin, *Roberts'* test for, 343
—— in semen, 308
—— in sputum, 84
—— in urine, 207
Siderosis pulmonum, 92
Silicates in sputum, 84
Simple sulphuric acid, 268
—— estimation of, 268
Sjöqvist's method for estimation of HCl, 101
Skatoxyl, 244
—— sulphuric acid, 244
Small-pox, protozoa in, 35
Smegma bacillus, 292
Soaps of lime and magnesia, 201
Soda solution, normal, 12
Sodium pyrophosphate, 270
Spasmotoxin, 34
Spectra, 38
Specific gravity of the blood, 4
—— gravity of the blood, estimation of, 5, 329
Spectroscope, *Browning's*, 43
—— double, 17, *note.*
—— lensless, 44
Spermatozoa, 188, 306
Spirals, 69
Spirillum of cheese, 137
—— of relapsing fever, 30, 191
—— of relapsing fever, spores of, 31
Spirochæta buccalis, 54
—— dentium, 54
Spores, preparation of, 324
—— staining of, 31, 139, 316
Sporozoa in fæces, 141
Sputum, the, 65
—— bacteria in, 74
—— blood in, 91
—— chemical examination of, 84
—— colour of, due to micro-organisms, 89
—— crystals in, 81
—— fatty acids in, 84
—— ferment in, 84
—— glycogen in, 84
—— in disease, 85
—— inorganic constituents of, 84
—— microscopical examination of, 66
Sputum, naked-eye characters of, 65
—— parasites of, 73
—— stratification of, 91
Staining fluids, preparation of, 75, 315, 337
—— of micro-organisms, *Ehrlich's* method, 75
—— *Gabbett's* method, 338
—— *Gram's* method, 28
—— *Giacomi's* method, 293
—— *Günther's* method, 31
—— *Friedländer's* method, 78
—— *Löffler's* method, 28
—— *Lustgarten's* method, 293
—— *Koch's* method, 75
—— *Wedl's* method, 295
—— *Weigert's* method, 295
—— with aniline dyes, 27, 75, 315, 337
Staphylococcus pyogenes albus, 54, 85, 91, 310
—— pyogenes aureus, 54, 292, 291, 310
—— pyogenes citreus, 54, 85, 310
—— pyogenes in milk, 308
Starch as a nutrient substance, 320
—— digestion of, 105
—— granules in fæces, 128
—— in fæces, 160
—— in gastric juice, 105
—— in urine, 205
Starvation, indican in, 242
Stas-Otto method, 117
Stearic acid, 162
Stercobilin, 126, 343
Sterilisation, methods of, 316
Sterilised blood-serum, 317
—— blotting-paper, 323
—— nutrient substances, 317
—— test-tubes, 316
Steriliser, 316, 322
—— steam, 317
Stewart on albuminuria, 342
Stokes's fluid, 41, 336
Stomach, atrophy of, 109
—— cancer of, 110
—— mucous catarrh of, 109
—— rate of absorption in, 106
Stomatitis, 58
Stratified sputum, 91
Streptococci, 33
Streptococcus erysipelatos, 33, 191
—— of scarlatina, 33
—— pyogenes, 33, 191, 310
Strongylides, 150
Strongylus duodenalis, 150
Succinic acid, 302
Sugar, grape, estimation of, 230
—— grape, estimation of, by fermentation, 232, 343
—— grape, estimation of, by polarisation, 233
—— grape, estimation of, by titration, 231
—— grape, estimation of, *Claude Bernard's* method, 336

Sugar, grape, estimation of, *Seegen's* method, 337
—— grape, in blood, 47
—— grape, in exudations, 299
—— grape, in urine, 224
—— grape, tests for, 225
—— fruit, *see* Lævulose
—— milk, 238
Sulphanilic acid test, 273
Sulphates of sputum, 84
—— of urine, 267
Sulphide of bismuth crystals, 157
—— of sodium solution, 254
Sulphocyanide of ammonium solution, 266
—— of saliva, 55, 57
—— of urine, 267
Sulphur in urine, 268
Sulphuretted hydrogen in blood, 42
—— hydrogen in fæces, 164
—— hydrogen in urine, 271
—— hydrogen poisoning, 42
—— hydrogen, tests for, 272
Sulphuric acid, compound, 242
—— acid, poisoning with, 111
—— acid, simple, 268
Syntonin in gastric juice, 104
Syphilis, bacillus of, 292
—— hæmoglobinuria in, 222
—— of liver, 279
—— peptonuria in, 219

Tænia cucumerina, 145
—— elliptica, 145
—— flavopunctata, 145
—— leptocephala, 145
—— madagascariensis, 145
—— mediocanellata, 142
—— nana, 144
—— saginata, 142
—— solium, 143
Tannin in urine, 288
—— test for ptomaines, 120
Tartrate of potassium and sodium, 231
Taurocholate of soda, 57
Teeth, deposit on, 59
Teichmann's crystals, 39
—— test, 39
Testicle-cells, 306
Test-meal, 104
Test-tube cultivation, 3
Test-tubes, sterilised, 317
Tetanin, 34
Tetanotoxin, 34
Tetanus, bacillus of, 34
Tetramethylene-diamine, 120, 263
Tetramethyl-paraphenyl-diamine, 102
Tetra-paper, 102
Thallin in urine, 173, 287
Thermostat, 324
Thiosulphates, 267
Thomas' cylindroids, 181, 187
Thoma-Zeiss' apparatus, 8
Thoracic duct, obstruction of, 301
Thread-worms, 150
Thrush fungus, 58, 73
—— fungus in fæces, 130
—— fungus in mouth, 58
—— fungus in nose, 64
—— fungus in stomach, 111
—— fungus in vagina, 310
Thymol test for chloroform, 121
—— test for sugar, 229
—— to preserve urine, 175
Titration method for estimating sugar, 230
Toluol, 248
Tongue, coating of, 60
Tonsillitis, 61
Tonsils, coating of, 61
Toxic nephritis, 281
—— states, urine of, 281
Transitory glycosuria, 224
Transudations, 301
Traube on elastic fibres, 69
Traube's corpuscles, 175
Trematodes, 147
Tribromophenol, 246
Trichina spiralis, 153
Trichinosis, 153
Trichocephalus dispar, 152
Trichomonas intestinalis, 142
—— vaginalis, 310
Trichotrachelides, 152
Trigeminal neuralgia, 56
Trimethylamin, 310
Triple phosphate crystals, 338
—— phosphate crystals in fæces, 157
—— phosphate crystals in pus, 298
—— phosphate crystals in sputum, 83
—— phosphate crystals in urine, 197, 202
Trommer's test, 48, 225
Tropæolin (00) test for HCl, 97
Troup on alveolar epithelium, 68
—— on spirals, 81
Trypsin, 264
Tubercle, bacillus of, 32, 75, 292
—— bacillus of, detection of, 75, 337
—— bacillus of, in blood, 27
—— bacillus of, in fæces, 139
—— bacillus of, in milk, 309
—— bacillus of, in pus, 292
—— bacillus of, in semen, 307
—— bacillus of, in sputum, 75
—— bacillus of, in urine, 191
—— bacillus of, spores of, 86
Tubercular infiltration of lung, 86
—— infiltration of lung, pneumonic type of, 86
—— infiltration of lung, typhoid type of, 86
Tuberculosis, chronic, 86
—— miliary, 86
—— of lung, 86
—— of urinary organs, 277
Tumours, fragments of, in fæces, 127

Tumours in urine, 188
Turpentine, oil of, 175
Typhoid fever, 167
—— fever, bacillus of, 33, 138, 191
—— fever, blood in, 33
—— fever, *Ehrlich's* urinary test for, 273
—— fever, fæces in, 167
—— fever, urine in, 273
Tyrosin crystals, 199
—— crystals in fæces, 156
—— crystals in sputum, 83
—— crystals in urine, 199
—— crystals, tests for, 200
Tyrotoxin, 121

Uffelmann's tests for HCl, 100
Ulcer, chronic gastric, 109
—— duodenal, 110
—— tubercular, 64
Ulceration, tubercular of urinary organs, 277
Ulcerative endocarditis, 191
—— enteritis, 166
—— rhinitis, 64
Ultramarine and zinc sulphide test, 100
Ultzmann's test, 239
Unna's blood-serum plates, 318
Unorganised casts, 180
—— sediment, 194
Uræmia, 51, 275
—— casts in, 181
Uranium solution, 270
Urates, casts of, 180, 181, 187
—— in urinary sediment, 201
Urea, estimation of, 257
—— excretion of, 278
—— in blood, 46
—— in fæces, 159
—— in gastric juice, 105
—— in saliva, 56
—— in urine, 256
—— tests for, 46
Urethra, epithelium of, 178
Urethritis, 277
Uric acid crystals, 195
—— acid diathesis, 253
—— acid, estimation of, 254
—— acid in blood, 47
—— acid in urine, 195
—— acid, tests for, 47
Urina spastica, 173
Urinary concretions, 202, 205
—— organs, tuberculosis of, 277
—— sand, 205
—— sediment, 175
Urine, the, 170
—— æther-sulphuric acids in, 242
—— albumin in, 206–224
—— amorphous deposit of, 201
—— bile acids in, 237
—— bile pigments in, 238
—— blood in, 222
—— carbohydrates in, 224
—— casts in, 180
Urine, chemical examination of, 205
—— chromogens of, 173
—— colour of, 172
—— crystalline deposit of, 195
—— detection of drugs in, 285
—— diacetic acid in, 250
—— diamines of, 263
—— epithelium in, 177
—— fat in, 251
—— fatty acids in, 251
—— fæcal substances in, 205
—— ferments of, 264
—— foreign bodies in, 205
—— fragments of tumours in, 188
—— gases of, 272
—— in anæmia, 280
—— in congestion, 273
—— in diabetes, 279
—— in diseases of alimentary canal, 278
—— in febrile states, 272, 343
—— infusoria of, 192
—— in hepatic affections, 278
—— in measles, 273
—— in nephritis, 274
—— inorganic constituents of, 265
—— in septicæmia, 191
—— in tuberculosis, 277
—— in typhoid, 273
—— microscopical examination of, 174
—— naked-eye inspection of, 170
—— organised sediment of, 175, 194
—— oxalic acid in, 252
—— parasites of, 189
—— proteids of, 205, 342
—— ptomaines in, 262
—— quantity of, 171
—— reaction of, 174, 341
—— sugar in, 224
—— urea in, 256
—— urobilin in, 240
—— vermes in, 193
Urinometer, 171
Urobilin, febrile, 343
—— in blood, 39
—— in fæces, 126, 163
—— in urine, 173, 240
—— normal, 341
—— of transudations, 302
—— spectrum of, 241
—— tests for, 240
Urobilin-jaundice, 240
Urobilinuria, 240
Urochrome, 173, 262
Uroerythrin, 173
Urotheobromin, 262
Uterine secretion, 310

Vaginal secretion, 309
Valerianic acid, 162
Vanillin, 98
Vapour-sterilisation apparatus, 317
Vermes in blood, 35
—— in fæces, 142
—— in pus, 298

Vermes in urine, 193
Vesical calculus, 277
—— epithelium, 178
—— tumours, 277
Vesuvin, 75
Vibrio buccalis, 54
Vicarious oxaluria, 253
Victoria blue, 315
Vierordt's spectrophotometer, 241
Vogel's colour scale, 174, 340
Volatile fatty acids in blood, 49
—— fatty acids in fæces, 160
—— fatty acids in gastric juice, 103
—— fatty acids in sputum, 84
—— fatty acids in urine, 251, 280
Volhard's method, 265
Vomit, the, 106
—— alcohol in, 121
—— alkalies in, 112
—— alkaloids in, 117
—— blood in, 109
—— carbolic acid in, 122
—— chloroform in, 121
—— contents of, 107
—— diamines in, 120
—— fæcal substances in, 111
—— fungi of, 108
—— hydrocyanic acid in, 123
—— in acute gastritis, 108
—— in cancer of stomach, 110
—— in chronic gastritis, 108
—— in gastric ulcer, 109
—— in parasitic affections of stomach, 111
—— in poisoning, 111
—— nitric acid in, 112
—— oxalic acid in, 112
—— ptomaines in, 119
—— pus in, 111
—— sulphuric acid in, 111
Vomitus matutinus, 108
Von Fleischl's hæmometer, 13
Von Mering and *Cahn's* method for estimation of HCl, 101
Vortmann's test for hydrocyanic acid, 124

Wasting diseases, indican in, 242
Waxy casts, 185
Weber's test for indican, 248
Wedl's litmus solution, 295
Weigert's staining process, 295
Weyl's test for kreatinin, 261
Whetstone crystals, *see* Triple phosphate
Whipworm, 152
White blood-corpuscles, *see* Leucocytes
Whooping-cough, bacillus of, 79
—— protozoa in, 79
Wool-sorters' disease, 30
Working eye-piece, 314, 347
Worm-Müller's test for sugar, 226
Worms, *see* Vermes
Würster's test for tyrosin, 200

Xanthin, 199, 205
Xantho-kreatinin, 260
Xantho-proteic test, 212
Xylol, 32

Yeast-Fungi of fæces, 130
—— of buccal secretion, 59
—— of sputum, 74
—— of urine, 189
—— of vaginal secretion, 310
—— of vomit, 108

Zeiss's lenses, 313, 346
Ziehl-Neelsen fluid, 77
Zinc sulphide and ultramarine test, 100
Zuntz' test for reaction of blood, 2
Zymogen of gastric juice, 95